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QUANTITATIVE ESTIMATION OF THE FIBROUS TISSUE IN PATHOLOGIC LIVERS

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SINCE neither liver function tests nor histologic studies gage accurately the extent of damage or of fibrosis of the liver, an attempt was made to establish a quantitative basis for the estimation of fibrosis as an index to the degree of permanent damage of the liver. In the development of alcoholic and some other types of cirrhosis there is initial reversible change, characterized chiefly by fatty infiltration, which is later followed by an irreversible stage, characterized chiefly by increased fibrosis. Thus the amount of fibrous tissue in the liver may be used to estimate the extent of damage and hence the prognosis in cases of cirrhosis.

In connection with studies of cirrhosis of the liver, therefore, it is desirable to obtain quantitative estimates of fibrous tissue for both normal and cirrhotic livers. The method is applicable to samples weighing as little as 0.4 Gm. and thus will be useful in determining the percentage of fibrous tissue even in pieces of liver obtained by peritoneoscopy.

MATERIAL AND METHODS

The material consisted of samples of liver tissue obtained at a series of 42 autopsies, which included 8 livers showing various stages of fibrosis and 34 noncirrhotic livers. The method for measuring collagen consists in extracting from a weighed amount of liver tissue substances other than collagen and elastin. No attempt was made to determine separately the amounts of collagen and elastin, as it seemed unnecessary for the purpose of this work. Different methods of extraction have been used by various workers.[1] In the present study the amount of fibrous tissue was determined by the method of Lowry, Gilligan and Katersky [2] as follows:

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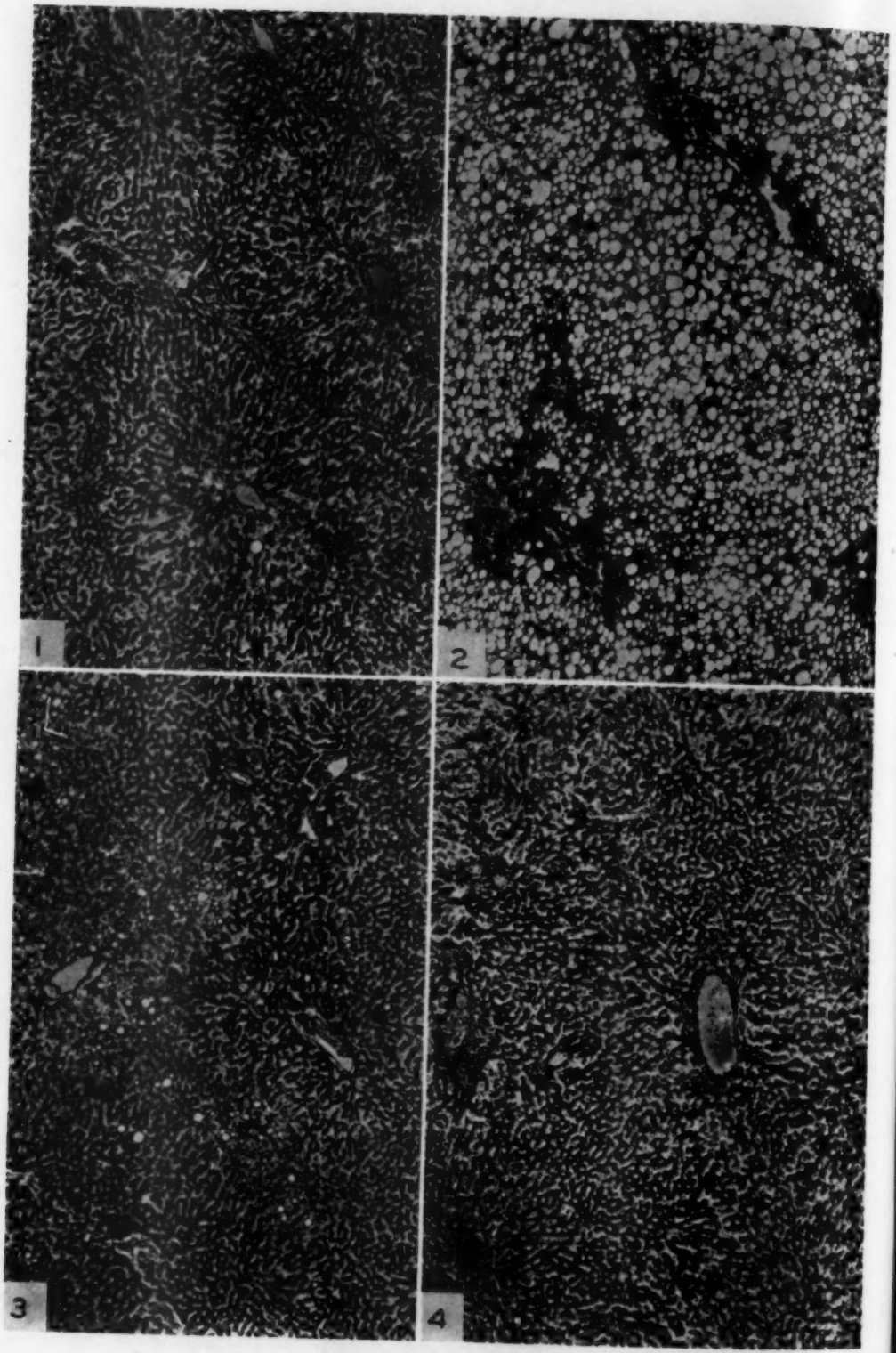
1. Schepilewsky, E.: Arch. f. Hyg. **34**:348, 1899. Mitchell, H. H.; Zimmerman, R. L., and Hamilton, T. S.: J. Biol. Chem. **7**:379, 1927. Spencer, H. C.; Morgulis, S., and Wilder, V. M.: ibid. **120**:257, 1937. Smith, E. C. B.: J. Soc. Chem. Indust. **54**:152, 1935. Hoppe-Seyler, G., and Lang, K.: Ztschr. f. physiol. Chem. **215**:193, 1935.

2. Lowry, O. H.; Gilligan, D. R., and Katersky, E. M.: J. Biol. Chem. **139**: 795, 1941.



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A 2 Gm. sample made up of portions of liver taken from different regions was used. In cases of early cirrhosis, two different samples were taken from the right and left lobes, respectively. The tissue was finely minced and then ground in a small porcelain mortar. It was rinsed into a 50 cc. round bottom, heavy pyrex centrifuge tube with tenth-normal sodium hydroxide until the total volume was about 40 cc. The mixture was stirred and allowed to stand for twenty-four hours. It was again stirred, centrifuged and the supernatant fluid pipetted off. Again 40 cc. of tenth-normal sodium hydroxide was added; the precipitate was stirred and allowed to stand for about two hours, with occasional stirring. It was again centrifuged and the supernatant fluid pipetted off. Forty cubic centimeters of water was then added, together with a drop of 0.1 per cent phenol red (i.e., phenolsulfonthalein) solution. The hydrogen ion concentration was adjusted to PH 7 (faint pink color) with tenth-normal hydrochloric acid. The preparation was centrifuged, and the supernatant fluid was removed. Forty cubic centimeters of a mixture of 3 parts of 95 per cent alcohol and 1 part of ether was added and stirred. It was allowed to stand for ten minutes and then was centrifuged. The supernatant fluid was removed by suction, and 40 cc. of ether was added; the mixture was stirred and centrifuged, and the supernatant fluid was pipetted off. The outside of the tube was wiped, and the tube with the material was dried in the oven at 100 C. for about four hours, i. e., to constant weight. It was cooled to room temperature and weighed or left in a desiccator until weighed (*A*). The dried material was then fixed in Zenker's solution, embedded and stained by Masson's trichrome technic, and microscopically examined to make sure that nothing but collagenous tissue had remained behind. The tube was then cleaned, dried and weighed (*B*).

Calculation: $\frac{A-B}{2} \times 100 =$ percentage of collagenous substances in the tissue.

Duplicate extraction was done in each case, and the mean of the two results was taken. Samples of liver tissue weighing 0.4 Gm. were used in some cases of cirrhosis with a fair amount of accuracy. As already pointed out, this procedure might be useful in determining the percentage of fibrous tissue in pieces of liver obtained by peritoneoscopy.

The result in each case was checked by a histologic study of the dried extracted tissue, to see if all the parenchyma and infiltrating cells, if any, had been dissolved out. Tissues were fixed in Zenker's solution and stained by Masson's trichrome technic, hematoxylin–eosin and eosin–methylene blue. Stained sections were studied with a view to establishing a possible correlation between the quantitative data and the histologic observations.

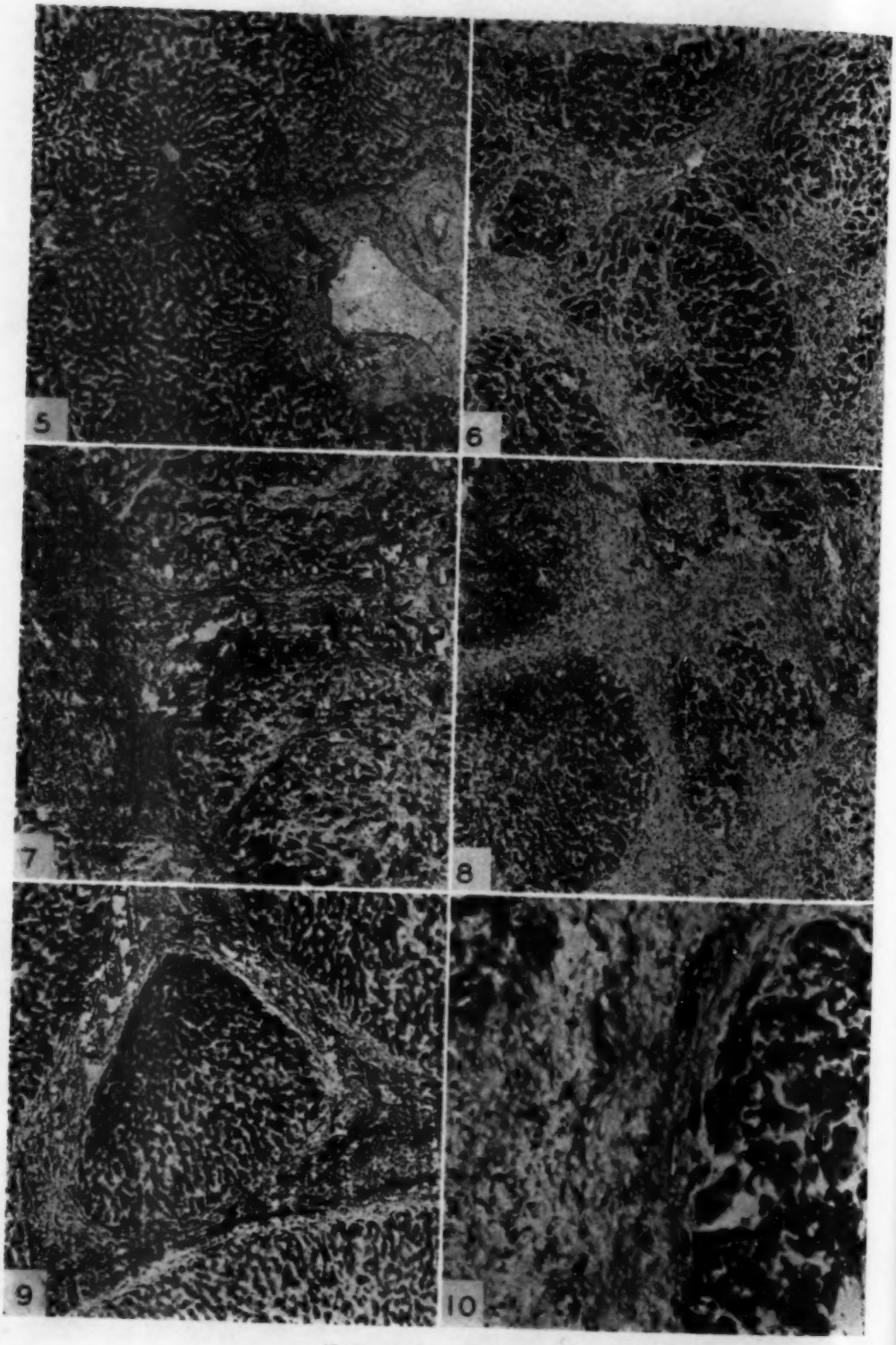
EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of liver with 1.5 per cent fibrous tissue. Hematoxylin and eosin stain; × 50.

Fig. 2.—Section of liver showing extensive fatty infiltration. The fibrous tissue content was 2 per cent. Hematoxylin and eosin stain; × 50.

Fig. 3.—Section of liver with 2.5 per cent fibrous tissue. Hematoxylin and eosin stain; × 50.

Fig. 4.—Section of liver with 2.8 per cent fibrous tissue. Hematoxylin and eosin stain; × 50.



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RESULTS

Fibrous Tissue Contents of Fibrotic Human Livers.—Samples of 8 human livers showing fibrosis were analyzed for fibrous tissue content. These livers included all grades of increase of fibrous tissue, from early to fully developed cirrhosis.

The table gives the percentages of fibrous tissue found for livers that were fibrotic as a result of various pathologic processes.

It is evident from the study of photomicrographs of livers showing different amounts of fibrous tissue that in the lower range of fibrous tissue content there is a fairly good correlation between the amount determined quantitatively and that seen histologically (figs. 1 to 7). With higher values it becomes difficult to judge the amount of fibrous tissue; i. e. figure 8, showing a section of liver with 13.3 per cent, and figure 9, showing one from a liver with 17.5 per cent, of fibrous tissue appear to show about the same extent of fibrosis.

Fibrous Tissue Contents of Fibrotic Human Livers

Liver	Histologic Observation	Percentage of Fibrous Tissue
1	Early portal cirrhosis	3.5
2	Portal cirrhosis	5.1
3	Portal cirrhosis	6.3
4	Portal cirrhosis	13.3
5	Portal cirrhosis	17.5
6	Healed yellow atrophy	23.0
7	Portal cirrhosis	20.2
8	Healed yellow atrophy	14.8

Fibrous Tissue Contents of Noncirrhotic Livers.—Thirty-four noncirrhotic livers comprising a wide variety of conditions but no primary or metastatic tumors were examined for their fibrous tissue content. The histologic examination did not show increases of fibrous tissue in the portal areas or in any other situation. The fibrous tissue content

EXPLANATION OF FIGURES 5 TO 10

Fig. 5.—Section of liver with 3.5 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 6.—Section of liver with 5 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 7.—Section of liver with 6.3 per cent fibrous tissue. Hematoxylin and eosin; × 50.

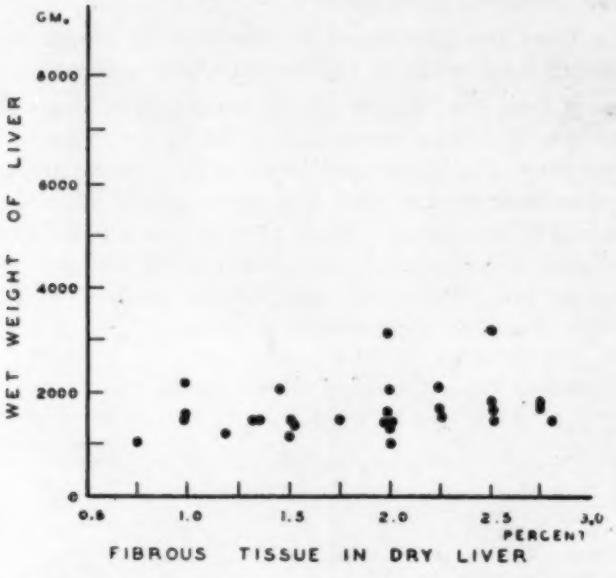
Fig. 8.—Section of liver with 13.3 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 9.—Section of liver with 17.5 per cent fibrous tissue. Hematoxylin and eosin; × 50.

Fig. 10.—Section of liver with 23 per cent fibrous tissue. Hematoxylin and eosin; × 50.

averaged 1.9 per cent of the dry liver weight, the range being from 0.8 to 2.8 per cent.

Figure 4 shows the histologic appearance of liver with 2.8 per cent of fibrous tissue. This is essentially normal liver.



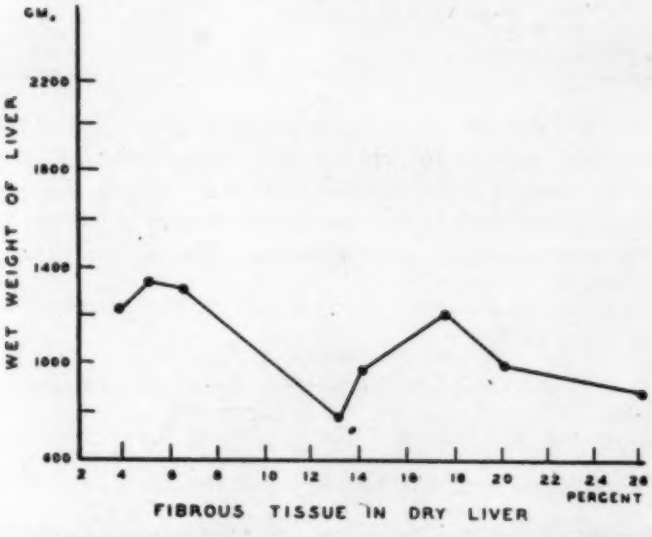


Fig. 11.—Weights of noncirrhotic livers with the corresponding percentages of their fibrous tissue contents.

Fig. 12.—Weights of fibrotic livers with the corresponding percentages of their fibrous tissue contents.

Weight of Liver and Amount of Fibrous Tissue.—Figures 11, 12 and 13 indicate the weights of noncirrhotic and cirrhotic livers and the corresponding percentages of fibrous tissue.

Figure 11 shows the weights and the percentages of fibrous tissue of noncirrhotic livers. It is clear that no relationship exists between the amount of fibrous tissue and the weight of the noncirrhotic liver. The outstanding examples were a liver which weighed 7,600 Gm. and had 1.5 per cent fibrous tissue, and another which weighed 1,010 Gm. and had 0.8 per cent fibrous tissue. The former was the liver of a person suffering from alcoholism and showed extensive fatty infiltration, which accounted for the increase of weight.[3]

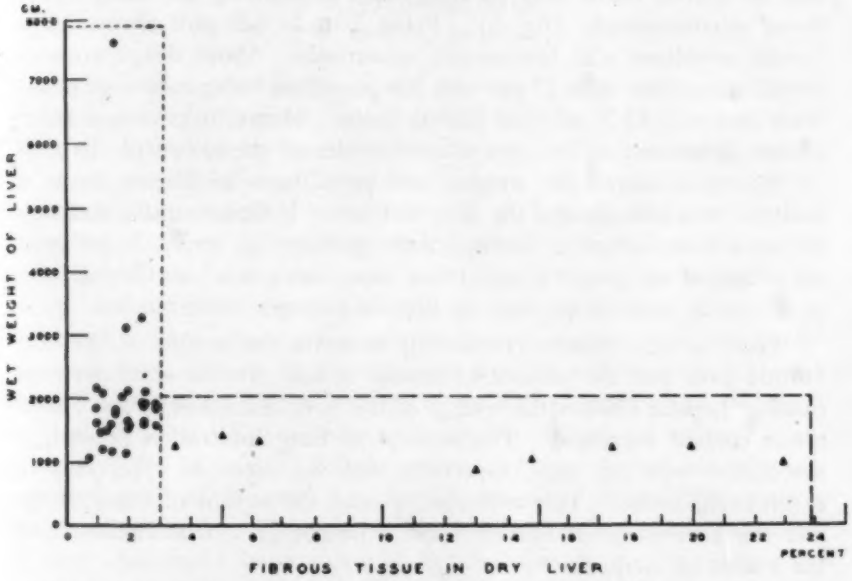


Fig. 13.—Weights of both the fibrotic and the noncirrhotic livers plotted with relation to their fibrous tissue contents.

Figure 12 shows the weights and the percentages of fibrous tissue of fibrotic livers. It clearly shows that there is a definite inverse relationship between the amount of fibrous tissue and the weight of the liver. As cirrhosis develops, the amount of fibrous tissue increases and the weight of the liver comes down.

3. While the stroma was apparently normal on the basis of the histologic examination, if the total liver weight was reduced to the normal of 1,500 Gm., the amount of fibrous tissue would be about three times the normal. This liver may well be precirrhotic.

COMMENT

The fibrous tissue contents of normal and fibrotic livers have been measured quantitatively. In livers which gave no evidence of hepatic damage, the average content was 1.9 per cent of the dry weight of the liver, the range being 0.8 to 2.8 per cent. In livers showing gross or microscopic evidence of fibrosis, the fibrous tissue content was from 3.5 to 23 per cent of the dry weight.

Histologic examination of liver sections showed that in the lower range of values of fibrous tissue there is fairly good correlation between the amounts of fibrous tissue determined quantitatively and the amounts seen in microscopic examination. For example, a liver with 3.5 per cent of fibrous tissue may be recognized as showing increased fibrous tissue microscopically (fig. 5). From 5 to 10 per cent fibrous tissue is well correlated with microscopic appearance. Above this, correlation is difficult, a liver with 23 per cent fibrous tissue being indistinguishable from one with 13.3 per cent fibrous tissue. Hence, microscopic examination alone may fail to give a correct idea of the extent of cirrhosis.

Figure 13 shows the weights and percentages of fibrous tissue of both the noncirrhotic and the fibrotic livers. It demonstrates that noncirrhotic livers show a fibrous tissue content of under 3 per cent, regardless of weight. The weights of those livers in which fibrous tissue is increasing tend to decrease as fibrosis becomes more marked.

There is no constant relationship between the weight of the nonfibrotic liver and the amount of fibrous tissue. In the small series of cases of hepatic fibrosis the weight of the liver decreased as the fibrous tissue content increased. The amount of fatty infiltration present, in association with cirrhosis, especially with the alcoholic type, may be a confusing factor. This will always send the weight of the liver up, and the percentage of fibrous tissue will not be a true indication of the extent of cirrhosis.

SUMMARY

The fibrous tissue contents of noncirrhotic, fibrotic and cirrhotic human livers have been measured by chemical means.

Good correlation was found between the amount of fibrous tissue microscopically visible and the weight of the liver in all cases of fibrosis except those in which fibrosis was extreme. As the amount of fibrous tissue increases, the weight of the liver decreases.

There was no apparent correlation between the amount of fibrous tissue and the weight of the liver in noncirrhotic cases.

PREINVASIVE CARCINOMA OF THE CERVIX UTERI

Seven Cases in Which It Was Detected by Examination of Routine Endocervical Smears

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INCIPIENT cancer of the cervix uteri is usually noninvasive, and when it is in this phase adequate therapy should result in cure in 100 per cent of the cases. In a statistical study of cases of cancer of the cervix, Pund and Auerbach [1] found that the average age of patients with preinvasive carcinoma was 36.6 years, six years below the average age of patients with covert invasive carcinoma and twelve years below that of patients with overt carcinoma. The detection of cancer which is in its preinvasive phase therefore offers a challenge to the physician. In general, preinvasive carcinoma gives rise to no symptoms and cannot be detected by physical examination. It can easily be missed in biopsy because of its limited extent and its endocervical location. The endocervical smear stained according to the method of Papanicolaou [2] offers the best routine method of detecting neoplastic cells of the cervix in their preinvasive phase; however, the diagnosis must be confirmed in biopsy or endocervical curettage. Several observers [3] have reported a few cases in which preinvasive carcinoma of the cervix was detected by examination of a vaginal smear. A study of routine endocervical smears has enabled us to report 7 additional cases.

This study was aided by a grant from the American Cancer Society.

From the Department of Pathology, University of Georgia School of Medicine.

1. Pund, E. R., and Auerbach, S. H.: J. A. M. A. **131**:960, 1946.

2. Papanicolaou, G. N.: J. A. M. A. **131**:372, 1946.

3. Meigs, J. V.: J. A. M. A. **133**:75, 1947. Ayre, J. E.; Bauld, W. A. G., and Kearnes, P. J.: Am. J. Obst. & Gynec. **50**:102, 1945. Meigs, J. V., and others: Surg., Gynec. & Obst. **81**:337, 1945. Papanicolaou, G. N., and Traut, H. F.: Diagnosis of Uterine Cancer by the Vaginal Smear, New York, The Commonwealth Fund, 1943. Fremont-Smith, M.; Graham, R. M., and Meigs, J. V.: New England J. Med. **237**:302, 1947.

MATERIAL AND METHOD

Smears from patients attending the various departments of the clinic of the University Hospital and from private patients of physicians of Augusta and surrounding towns were submitted to this department for examination. The method used for this study is simple and requires little time and effort on the part of the physician. An ordinary cotton applicator is inserted into the cervical canal, twirled a few times in one direction and then rolled on a slide. In everted cervices the applicator should also be rubbed against the area of eversion. In addition a smear is made from the vaginal pool for study of endometrial cells and for study of the cytologic changes which reflect hormonal activity. For vaginal films a speculum is not required. The applicator is inserted deeply into the vagina, twirled and rolled on a slide.

The slides are immediately immersed in a solution of equal parts of ether and 95 per cent alcohol and should remain in this solution for at least fifteen minutes

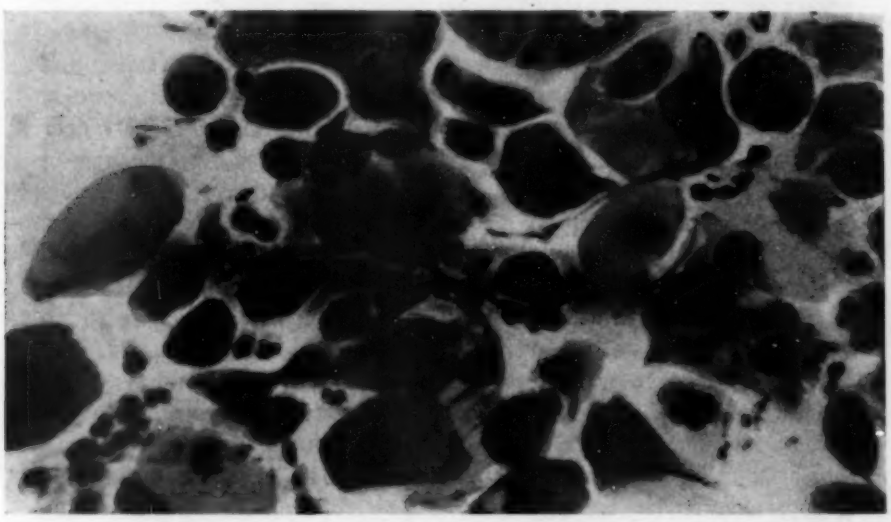

Fig. 1 (case 2).—Endocervical film. The nuclei are hyperchromatic, disproportionately large and irregular in size and shape. Compare the abnormal cells with the one normal cornified cell showing a small pyknotic nucleus.

but may remain there for several hours. If the slides are to be sent to the laboratory, drying is prevented by the technic recommended by Ayre.[4] One or two drops of glycerin should be placed on the film, and it is then covered with a clean slide, or two filmed slides are placed face to face. The endocervical smears are stained by the method of Papanicolaou,[2] which affords the greatest transparency and the clearest nuclear detail. Vaginal smears are stained for glycogen with hematoxylin and with Best's carmine.

REPORT OF CASES

Case 1.—The patient was a white woman aged 72 years, a decipara. Cervical biopsy and curettage of the uterus were performed at the time of perineorrhaphy.

4. Ayre, J. E.: South. M. J. **39**:847, 1946.

Clusters of cells suggestive of cancer were found in the stained endocervical films which had been secured before operation. In a biopsy specimen removed from the cervix preinvasive carcinoma was observed in one of the glands. This patient is being treated with radiation.

CASE 2.—A Negro woman aged 44, a unipara, complained of slight vaginal bleeding of six weeks' duration. Definite cancer cells were observed in vaginal and cervical films (fig. 1). A cervical specimen was obtained for biopsy, and the endocervix was curetted. The microscopic diagnosis was: "Chronic inflammation of the junctional endocervix of the small section of the cervix. The scrapings are from the endocervix, and among the fragments of normal squamous and glandular epithelium there are three shreds of squamous carcinoma which are noninvasive" (fig. 2). Three weeks later total hysterectomy was performed. Although in serial blocks taken from the junctional endocervix no definite cancer was found, regenerating squamous epithelium, in which there were foci of anaplasia, had begun to repair the denuded surface.

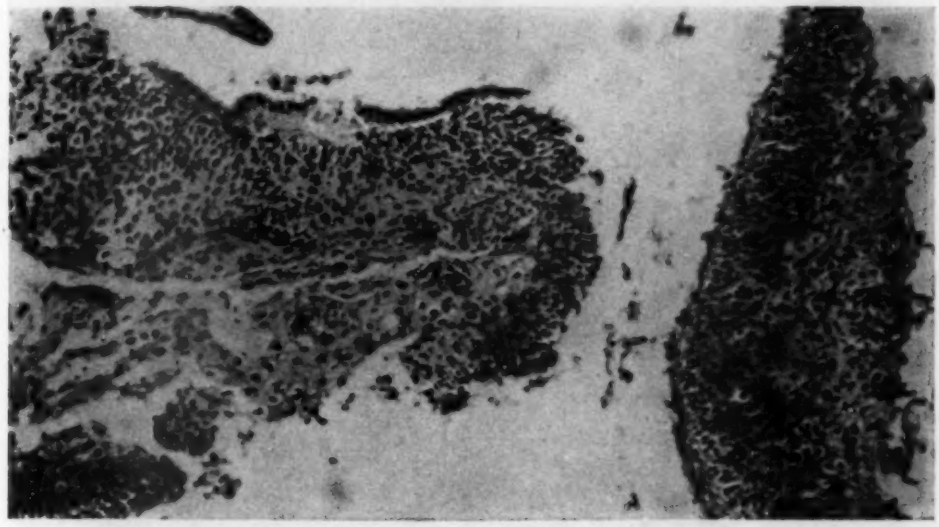

Fig. 2 (case 2).—Two shreds of cancerous squamous epithelium curetted from the endocervical canal. Note the irregularity in size of nuclei and the lack of differentiation of cells. The shreds of epithelium are limited by a basement membrane. Fragments of normal columnar epithelium are included in the curettings.

CASE 3.—A white woman aged 38, a quadripara, was referred to the cancer clinic because of irregular and excessive uterine bleeding. Few cells suggestive of cancer were found in the cervical film (fig. 3*A*), but they were definitely abnormal. Preinvasive carcinoma was observed in three of the four sections of cervix (fig. 3*B*); no carcinoma was seen in the endocervical curettings. This patient received 2,000 milligram hours of radium therapy, and hysterectomy was subsequently performed. No remains of cancer were found in serial blocks of the junctional endocervix.

CASE 4.—The patient was a white woman aged 32 years, a tripara. Abnormal cells were found in the endocervical film which was made by a private physician for routine study. Preinvasive carcinoma was found in one of three small sections

of cervix. No carcinoma was found in the endocervical curettings. At the time of the biopsy the cervix was extensively cauterized. After subsequent hysterectomy, no remains of the carcinoma were found in serial blocks of the cervix.

CASE 5.—A Negro woman aged 35, a tripara, complained of bleeding between periods for five months. On vaginal examination a superficial erosion was observed at the os of the slightly enlarged cervix. In two sections of the biopsy specimen of the cervix, preinvasive carcinoma was found, which involved the surface and the mouths of the glands. Over a period of a month the patient received 3,000 roentgens of high voltage roentgen radiation. Six weeks later, on vaginal examination, the cervix appeared normal. In the endocervical smear, however, cancer cells were observed as well as minor radiation changes. Total hysterectomy

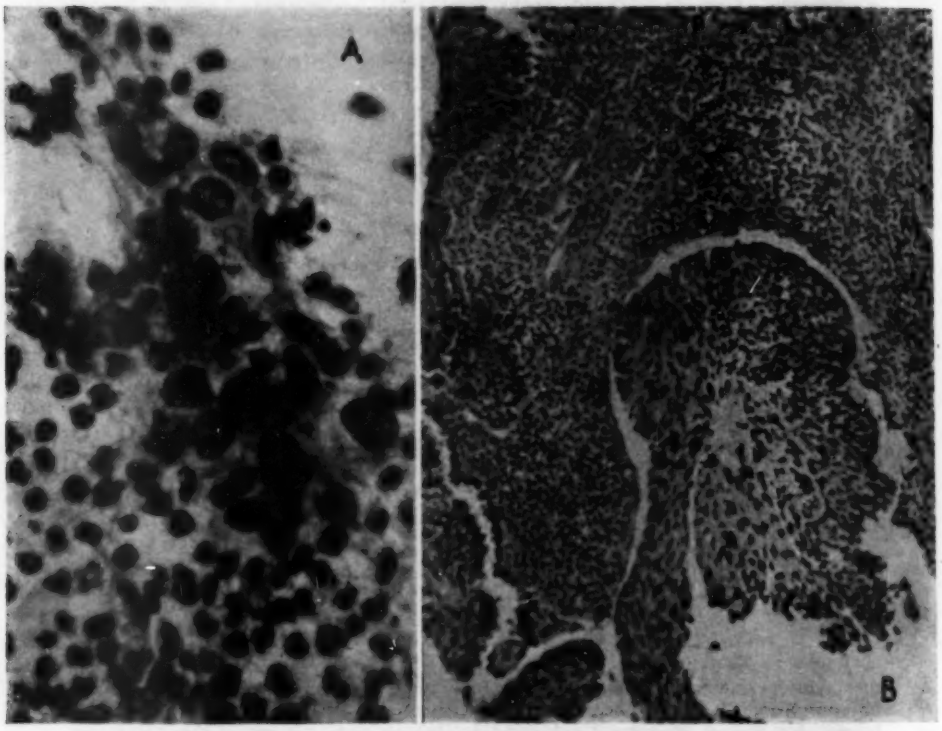

Fig. 3 (case 3).—*A*, endocervical film. Only a few atypical cells were observed. The nuclei are disproportionately large, vesicular, hyperchromatic and of varying size and shape. *B*, biopsy specimen. Undifferentiated squamous epithelium is limited to the surface and extends into the mouth of one gland.

was performed. A minute preinvasive carcinoma was found at the junctional endocervix in only one of several serial blocks.

CASE 6.—A white woman aged 33, a tripara, complained of leukorrhea. In the routine endocervical film definite cancer cells were found. Biopsy and endocervical curettage were performed. Preinvasive carcinoma was found in two of the three small sections of cervix, and slivers of cancerous epithelium were observed in the endocervical curettings. No invasion was seen. Total hysterectomy

was performed five weeks later, and carcinoma was present in two of ten blocks of junctional endocervix (fig. 4).

CASE 7.—A white woman aged 62 years, a secundipara, had noted "spotting" on one occasion. On vaginal examination, the cervix was seen to be everted, and the anterior lip at the site of eversion appeared thickened and velvety. A small polyp projected from this lip, and an additional small polyp was present on the posterior lip. In the endocervical film, which in this case was prepared by scraping the endocervix with a wooden applicator, there were numerous shreds of atypical squamous epithelium. The nuclei were hyperchromatic, disproportion-

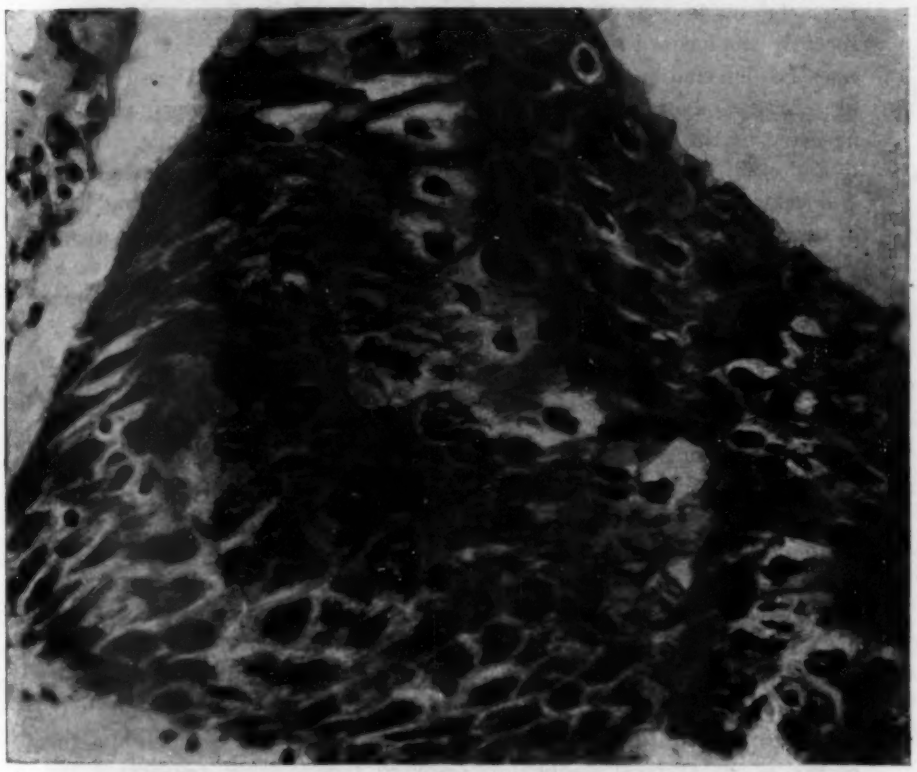

Fig. 4 (case 6).—High magnification of preinvasive carcinoma found in a section of cervix after total hysterectomy. The presence of this carcinoma was suggested by an endocervical film, and the neoplasm was demonstrated in a biopsy specimen. The cells are distinctly neoplastic, although there was no invasion. The nuclei are vesicular, hyperchromatic, disproportionately large and irregular in size. Cellular differentiation is incomplete.

ately large and variable in size, and numerous mitoses were seen. There was no evidence of polarity. Free atypical cells were also observed. The cells were so characteristic of cancer that biopsy was recommended, and radium therapy was instituted at the time of biopsy. A generous biopsy specimen of the anterior lip included the attached polyp, and the other polyp was removed. No curettings could be obtained from the body of the uterus. In two of eight microscopic sections of the biopsy specimen small foci of preinvasive carcinoma were observed. Much of the surfaces had been denuded by the applicator and by surgical preparation.

COMMENT

Our observations confirm those of others that preinvasive carcinoma can be detected by the examination of vaginal and endocervical spreads. The presence of atypical cells, however, is not always indicative of cancer. We wish, therefore, to emphasize that it is necessary to confirm the diagnosis by histologic sections. Biopsy of two or more sites should be carried out and care exercised to choose the proper sites—the endocervical junction at the external os. Due allowance must be made for variations when ectropion is present. We have recently emphasized the importance of endocervical curettage.[5] Cancers may be detected in this manner when not found in biopsies (case 2, fig. 2). Conversely, however, the endocervical scrapings may be negative in the presence of a positive cervical biopsy specimen (cases 3 and 4). It is therefore recommended that biopsy specimens and endocervical curettings be obtained. Since we are recommending total hysterectomy without ovariectomy for the younger age group of patients with preinvasive carcinoma, the endocervical curettage also serves to differentiate the preinvasive carcinoma from covert invasive carcinoma.

In our study we prefer at the present time the swab method of obtaining the smear from the endocervix. The junctional endocervix is generally the site of the incipient cancer, and we assume that more cancer cells would be found there than in the vaginal smears. However, vaginal smears should also be made at the same time for the study of the cytologic changes which are caused by the hormones and also for the detection of abnormal endometrial cells. With the swab method, however, cancer cells are not found in large numbers, and careful prolonged examination is necessary. Normal cells constitute the chief cells of the film, and only a few small foci of cancer cells may be observed. For our purpose we preferred the cotton swab rather than a scraping instrument. We initiated this study for the detection of preinvasive cancers, and we desired confirmation with histologic preparations. We therefore feared the risk of denuding the cervix of its atypical epithelium prior to biopsy. By preserving the surface epithelium we have also been able to explain from subsequent biopsies and curettage some of the "suspicious" films. The chief offenders of this group we found to be healing ulcerations, imperfect metaplasia in estrogenic deficiency and metaplastic epithelium with superficial maceration.

It has been demonstrated that some preinvasive carcinomas can be detected by vaginal and endocervical smears. Further study however

5. Pund, E. R., and others: Preinvasive and Invasive Carcinoma of Cervix Uteri: Pathogenesis, Detection, Differential Diagnosis and the Pathologic Basis for Management, Am. J. Obst. & Gynec., to be published.

is necessary to determine whether all cancers of the cervix can be diagnosed in their incipiency. When we have accumulated sufficient material, we shall attempt to analyze our results and compare the incidence with that of Pund and Auerbach,[1] who reported that preinvasive carcinoma occurred in 3.9 per cent of 1,200 surgically removed cervices. Because the incipient cancers are frequently asymptomatic and grossly invisible, the making of vaginal and endocervical smears should be a routine procedure in physical examinations, especially in those of parous women. In this way a larger number of cervical carcinomas will be diagnosed in their curative stage.

SUMMARY

Seven cases in which preinvasive carcinoma of the cervix was detected by study of endocervical smears are reported.

The importance of making routine endocervical smears for the detection of incipient cancer of the cervix is discussed.

In cases in which the smears show cancer cells or cells arousing suspicion of a cancerous change, the diagnosis of cancer of the cervix should be confirmed by biopsy.

The value of endocervical curettage is emphasized.

RARE ANOMALY OF THE VERMIFORM APPENDIX

Mucous Lining of the External Surface

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DISTURBANCES of development consisting in improper lodgments of elements of mucous membrane usually concern the female generative organs. There is displacement of larger or smaller clusters of endometrium—endometriosis; seldom seen is the group of endometrial cells displaced to the peritoneal uterine covering, called endometriosis externa, or the cul-de-sac, occasionally encountered on the broad or the round ligament.

Rarely observed are transplantations of elements of other mucous membranes, as elements of the intestinal tract misplaced in the outer wall lining—enterocystoma—and usually considered as remnants of the fetal circulation—ductus omphalomesentericus—or of the pancreatic tissue. Spreading in the intestinal wall, the glandular elements may lodge under the peritoneal cover or in the muscular layer of the intestine, causing glandular enlargements—adenomyomatosis. Such changes may simulate a new growth, giving cause for surgical intervention.

Rare are the observations pointing to elements of the intestinal inner lining displaced to the peritoneal surface: The formation of a membrane on the surface of a peritoneal coat may have an inflammatory origin. The place at which such changes most frequently occur on the intestinal tract is the appendix.

The reporting of the case observed by me is worth while not only because a membrane, tunica mucosa, was abnormally placed and formed on the outer surface of the appendix but because it covered the entire appendix, giving an impression of a normal appearance and suggesting numerous questions as to the nature of its development and the associated pathologic condition. In the literature that was available to me, I could not find any description of such a displacement of mucous membrane.

A man aged 28, a policeman, was admitted to St. Jacob's Hospital, Vilna, Poland, May 2, 1942. He was well built and well nourished. He complained of pain in the abdomen and slight nausea. The history and the physical findings were essentially irrelative. His temperature was 99.6 F. A diagnosis of subacute

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appendicitis was made, and after induction of local anesthesia, the appendix was removed. There were no complications during the operation, and no adhesions were found. The postoperative course was uneventful. The incision healed by first intention, and the patient left the hospital on the tenth day, in good condition. So far the clinical picture is that of the common subacute appendicitis. The removed appendix was sent to the histopathologic department.

Macroscopically, the specimen was of normal thickness and about 10 cm. long. The surface appeared somewhat velvety and rather pale. The tip was slightly inflamed and the attachment of the mesoappendix well defined.

Microscopically, under low power, the cross section readily revealed an abnormal construction, namely, the placement of mucous membrane, instead of the serous coat, as the outer lining of the appendix. It was really a second mucosa, differing in no way from that lining the inside of the appendix. The external mucosa covered all of the appendix except the mesoappendix. Also with low power it was plainly seen that the glands of the outer lining penetrated the wall of the appendix, forming a junction between the inner and outer linings, and the glands

Cross section of the appendix; × 60: (*a*) Normal mucosa. (*b*) Mucosa as outer coat. (*c*) Juncture of both mucous membranes. (*d*) Place at which mesoappendix was attached.

of both the inner and the outer mucosa were of the same type. The covering layer of these glands consisted of cylindric cells. These cells were swollen and evenly distributed over the basic membrane. A large number of cuboidal cells, filled with cytoplasm, gave the picture of active mucosal cells. The nuclei of the cells were well visualized and normally placed. There were a few erythrocytes attached to the cell membranes. Between glands the texture was edematous; in the outer layers were minute hemorrhagic spots. The arterioles were dilated, and numerous absorbing cells were seen. In the wall of the mucosa, between the glands, many granules were noted; some were close to the surface of the mucosa, while others, in groups, were penetrating into the deeper layers.

The submucosa was thin: The muscle cells were well defined, but the inner layer was not so well developed. The submucosa of the normally placed mucous membrane was wide, containing many dilated blood vessels, also absorption spaces. The basement membrane was of a hemorrhagic type. Numerous sections of the glands contained many cuboidal cells in active condition. The stroma or tunica

propria contained many squamous cells. In the lumen of the appendix, and also on the outer covering, were seen clusters of erythrocytes.

Changes in both mucous membranes (the dilated blood vessels and the free erythrocytes) speak for subacute inflammation. These changes were alike in both mucosae, the inner and the outer.

The anomalous mucous membrane must have formed over the appendix during embryonic life, before the formation of the primary intestinal tract. It is important to remember that during the evolutionary stages the elements of mucosa lodged in the place of the serosa continued to grow normally in the early development of the fetal life, giving the picture of normal mucosa. That mucosa, placed over the outer surface of the appendix, developed fully, regardless of the serosa of adjacent structures and the entirely different function of its natural habitat, and retained the formation and consistency of intestinal mucosa for a period of twenty-eight years. In spite of the inflammatory condition observed, the surrounding tissues did not react adversely, as no adhesions were found during operation.

Although this report does not present an exhaustive picture, as it concerns only the biopsy aspects of the question, and nothing is known about the condition of the serous membranes adjacent to the appendix, it does point to the possibility of development and existence of a considerable extent of mucosa of the digestive tract in an atypical place.

PRIMARY ATYPICAL PNEUMONIA

Report of Eight Cases with Autopsies

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ALTHOUGH cases of primary nonbacterial pneumonia of unknown but probably viral cause have been recognized in great numbers during recent years,[1] in most of them the disease has been mild or of only moderate severity. Fatal cases have not been frequent, and only a few descriptions of the pathologic changes in such cases are available.[2] A group of 8 such cases in which autopsies were done is therefore of interest. In all these cases there was a characteristic clinical course with similar physical, roentgenologic and pathologic findings in the lungs and, in addition, there were cold agglutinins in the serum. All but one of them occurred in the latter months of 1942.

REPORT OF CASES

CASE 1.—This case is reported in more detail elsewhere because the pneumonia was associated with a bullous type of erythema multiforme.[3] The illness in this 17 year old boy began on Aug. 12, 1942, with coryza and malaise, followed in a week by a cough productive of gray sputum. On August 25 he had a chill and began to have a sore throat, dysphagia, blood-streaked sputum and pain in the anterior region of the chest. The course of the illness was characterized by an extensive bullous eruption of the skin and of the mucous membranes of the orificial surfaces, severe conjunctivitis and the symptoms, signs and roentgenologic findings

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of diffuse atypical pneumonia. Oxygen and sulfadiazine and tube feedings with vitamin supplements were of no avail. The patient became increasingly dyspneic, cyanotic and delirious and died on September 3. The only significant laboratory findings were: white blood cell counts ranging mostly between 12,200 and 17,500, with 85 to 60 per cent polymorphonuclear neutrophils; sputum cultures which showed alpha hemolytic streptococci in varying numbers and Staphylococcus aureus in increasing numbers; a psittacosis complement fixation titer of 1:256 (4 plus) for the serum on August 28 and September 3 and a cold agglutinin titer of 1:640 on the latter date.

Autopsy (eighteen hours after death).—Over the entire body there were lesions varying from 0.5 to 2 cm. in diameter; some were blebs which contained clear fluid, but many were dried, and others, including those on the lids, the scrotum and the penis, appeared ulcerated and covered with hemorrhagic crusts. There was congestion of the conjunctival vessels, with some conjunctival hemorrhages.

The gross appearance of the lungs is shown in figure 1. The combined weight of the lungs was 1,200 Gm. There was a thin layer of fibrin over the lower lobe of the right lung and over both lobes of the left lung. The middle lobe was subcrepitant and had a white surface, but all the other lobes of the lungs showed marked decrease to absence of crepitation and were purple-red. The cut surfaces were dark red-purple, and no purulent material could be expressed; they presented an appearance of miliary nodules and showed some dark areas of congestion and atelectasis, especially in the posterior and dependent portions. The mucosa of the trachea and bronchi was congested. The tracheobronchial lymph nodes were not enlarged. There was some congestion of the cerebral veins. The remaining organs appeared grossly normal.

Microscopic Examination.—The observed changes varied greatly in different areas of the lungs. In some areas the alveoli were essentially normal; in others they contained an albuminous precipitate, and in still others there were fibrin and red blood cells. The lining cells of many alveoli were swollen and vacuolated, and a few showed mitotic figures. In such areas the exudate in the alveoli consisted of desquamated alveolar lining cells, plasma cells, other mononuclear cells, an occasional multinucleated cell and a rare giant cell of the foreign body type (fig. 2 *B*). In some places the bronchi contained numerous polymorphonuclear leukocytes and some clumps of cocci (fig. 2 *A*). The peribronchial and perivascular regions were infiltrated by plasma cells, some lymphocytes, rare mast cells and eosinophils, and some large immature cells of an unidentified type. In the submucosa of the trachea and about the glands there was an infiltration in which lymphocytes, many plasma cells and an occasional mast cell took part.

A section of the skin showed a vesicle with necrosis of the covering epithelium and a base consisting in part of intact epithelium and in part of necrotic connective tissue infiltrated by polymorphonuclear leukocytes and large mononuclear cells. The vesicle contained precipitated albumin and fibrin in one part and polymorphonuclear leukocytes, large mononuclear cells, fibrin and a moderate number of cocci in another. Some of the hair follicles and coil glands were necrotic. In the deeper portions of the corium there was a perivascular infiltration of large mononuclears, lymphocytes, plasma cells and an occasional eosinophil and mast cell. The blood vessels in the necrotic connective tissue were thrombosed.

There were a few scattered large mononuclear cells, lymphocytes and plasma cells in the cerebral meninges, and similar cells infiltrated the interstitial tissues of other organs.

Bacteriologic Study.—Cultures of the heart's blood showed no growth. Hemolytic Staph. aureus was cultured from the lower lobe of the right lung, the lower

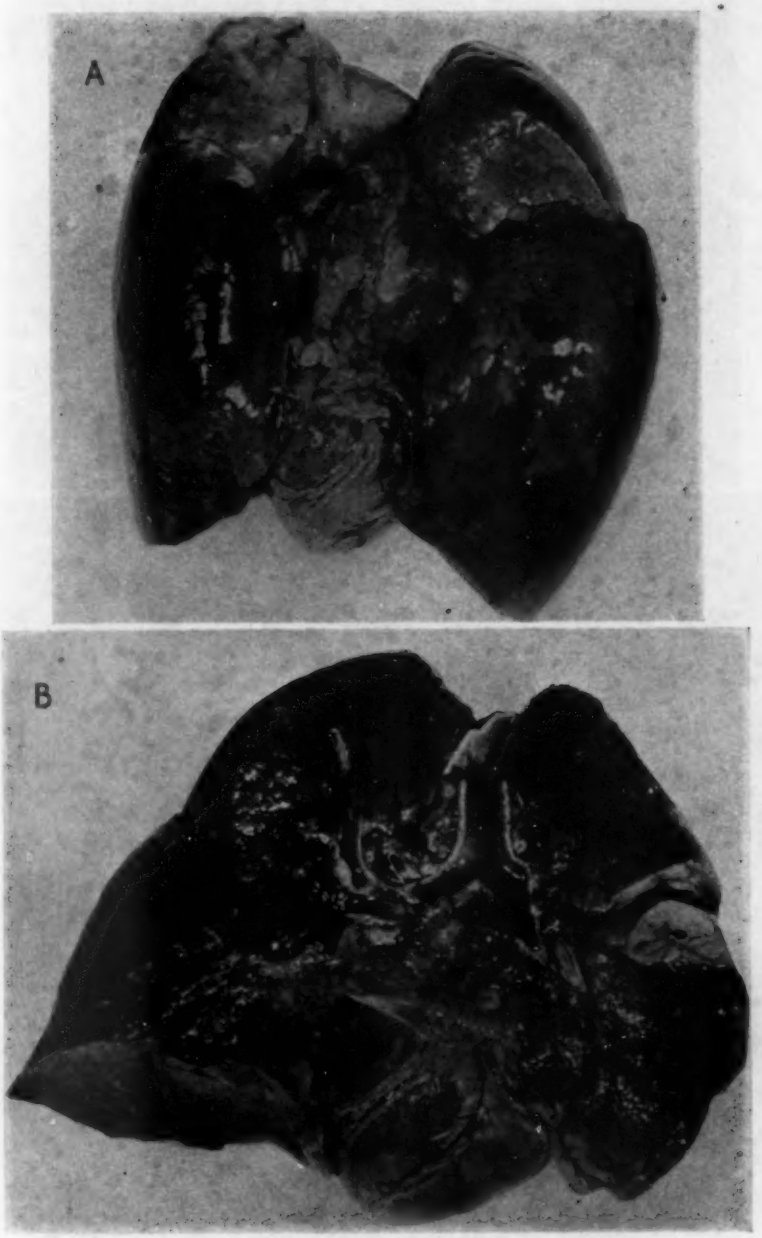

Fig. 1 (case 1).—*A*, posterior view of the lungs, showing the sharply demarcated dark areas of atelectasis of the lower lobes and the pale gray surfaces of the emphysematous upper lobes. (The shiny white areas are high lights.) *B*, cross section of these lungs. The cut surfaces show numerous small gray nodules, surrounded by darker congested areas, in the lung parenchyma. The mucosa of the trachea and bronchi is deeply injected. (The white areas are high lights.)

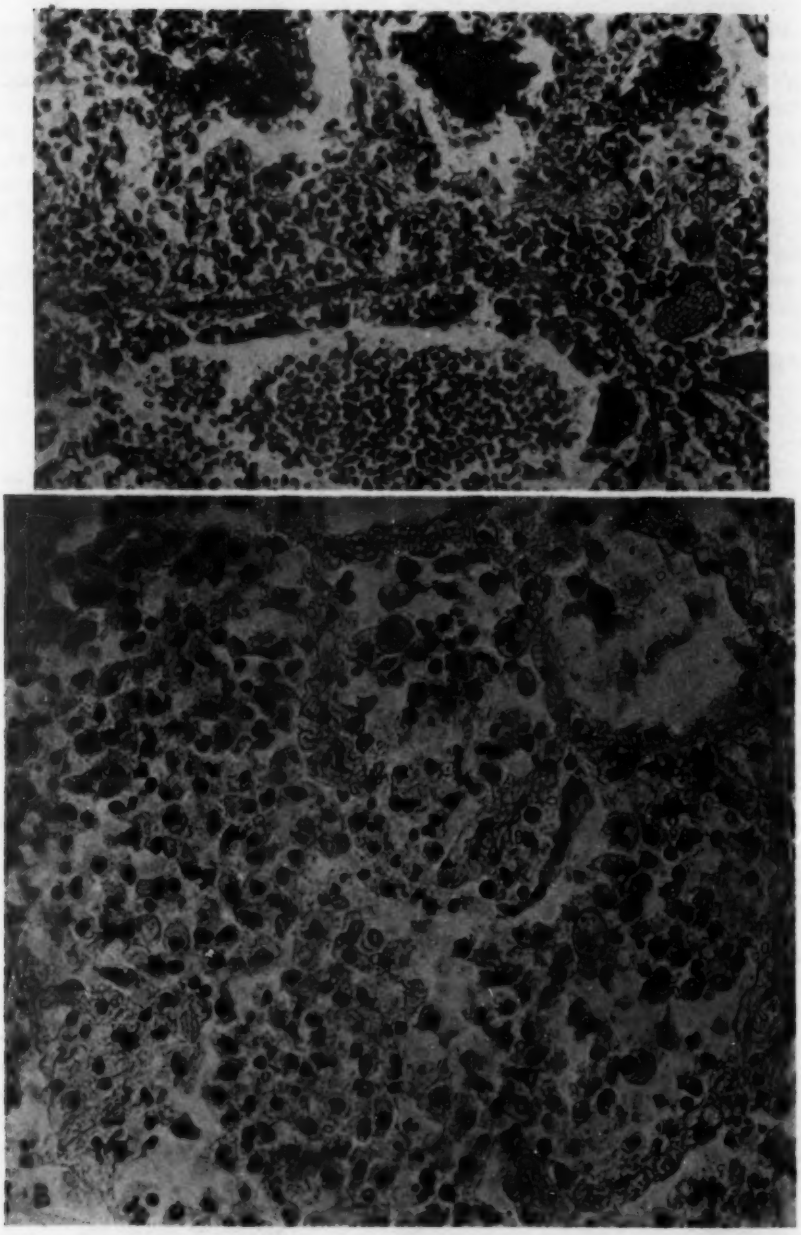

Fig. 2 (case 1).—*A*, bronchiole containing polymorphonuclear leukocytes in its lumen. Its wall is infiltrated with mononuclear cells. The adjacent alveoli contain fibrin and large mononuclear cells. Phloxine–methylene blue stain; × 100.

B, alveolar lumens containing an exudate of mononuclear cells. Phloxine–methylene blue stain; × 400.

lobe of the left lung, the pleura and the liver, and the last contained an enterococcus in addition. Mice and embryonated eggs inoculated with vesicle fluid and suspensions of the lungs have yielded no virus that could be identified.

CASE 2 (courtesy of Drs. Henry D. Stebbins and D. A. Nickerson).—A 50 year old white married woman began to have chilly sensations and general malaise on Sept. 27, 1942. On the following day she had a nonproductive cough, and on each of the next two days she had shaking chills, each followed by a rise of temperature to 103 F., but her physician found no abnormal signs in the chest. He sent her to the Salem Hospital on September 30. There was a vague history of mild asthmatic attacks in the past. A few years previously she had some urinary symptoms, but studies at that time revealed normal renal function.

At the hospital physical examination gave essentially negative results except for the fever. The hemoglobin content was 86 per cent; the red blood cell count, 4,160,000; the white cell count, 4,900, with 68 per cent polymorphonuclear neutrophils. The initial examination of the urine showed albumin (3 plus), with a specific gravity of 1.013 and a normal sediment. The blood nonprotein nitrogen was 42 mg. per hundred cubic centimeters. A blood culture was negative. Roentgenograms of the chest showed diminished radiance in the right lung field with linear, mottled and patchy densities in the lower half of this lung and a few small nodular areas on the left.

The patient was given sulfathiazole, 2 Gm., followed by 1 Gm. every four hours, together with an equal amount of sodium bicarbonate. This was discontinued on October 2 because of increasing vomiting, restlessness and headache and finally the appearance of a maculopapular rash. On October 10 the patient was given sulfadiazine, 1 Gm. every four hours, and on October 12, because of low blood concentrations, she received an intravenous injection of 5 Gm. of sodium sulfadiazine in a liter of 5 per cent dextrose. This was repeated twelve hours later. No further sulfonamide therapy was given. The fluid intake, part of which was given parenterally, ranged from 2,500 to 5,000 cc. per day, and the urinary output corresponded. The patient was kept in an oxygen tent continuously after October 2 except for a few occasions when pure oxygen was given by mask in attempts to relieve severe dyspnea. Aminophylline, in doses of 0.5 Gm., and epinephrine, 0.2 to 0.5 cc. of a 1:1,000 solution, were given for dyspnea that was associated with wheezing. Codeine, barbiturates and occasional small doses of morphine were given as sedatives. On October 11 a plasma transfusion was followed by a severe chill. After October 12 she received daily transfusions of 250 to 500 cc. to a total of 2,500 cc.

The patient's course was a stormy one. For the first ten days in the hospital, the fever was irregularly sustained, with peaks of 102 to 104 F., the pulse rate ranged between 100 and 120 and the respiratory rate between 25 and 30 per minute. There were three severe chills during this period. On October 11 the temperature dropped and stayed below 101 F., but the pulse rate and respiratory rates rose. The blood pressure was 140 systolic and 80 diastolic and gradually dropped to 110 systolic and 60 diastolic. Dyspnea and cyanosis increased steadily, with periods of asthmatic wheezing and severe labored breathing, which were only partly relieved by epinephrine and aminophylline. Rales appeared in the lungs soon after entry and increased until both lungs were filled with medium and coarse crepitant rales, and there were scattered musiçal rales during the periods of air hunger. No definite areas of consolidation were made out, however. The patient became increasingly stuporous and was unconscious during most of the latter half of her stay in the hospital. She died on October 18.

The white blood cell count was 4,700 on October 2, but the polymorphonuclears rose to 85 per cent. On October 5 the leukocyte count rose to 7,900, then to 24,100 on October 9, and thereafter ranged from 25,000 to 32,000, with 92 to 96 per cent polymorphonuclears, most of which were young forms and contained toxic granules. On October 9 the scleras were noted to be icteric, and the icterus index on that day was 14. On October 12 the hemoglobin content was 44 per cent, and on the following day it was 37 per cent, with a red blood cell count of 1,520,000. After transfusions the hemoglobin rose steadily to 84 per cent and the red blood cell count to 3,780,000. The urine, examined at four day intervals, showed a specific gravity of 1.009 to 1.013 and albumin (2 to 3 plus), with a few white blood cells and rare red blood cells in the sediment. On October 8 a test of the urine for bile was recorded as positive, and there was an unusually large amount of albumin in the same specimen. The blood nonprotein nitrogen stayed between 32 and 44 mg. per 100 cc. until October 10; the following day it rose to 75 mg. and thereafter fluctuated between 80 and 130 mg. The blood sulfathiazole level on October 2, just before that drug was stopped, was 6 mg. per 100 cc. Sulfadiazine levels on October 10 and 12 were 5 and 13 mg. per 100 cc., respectively. Serum proteins ranged from 5.1 to 5.9 Gm. per 100 cc. The stools were negative for occult blood. On October 10 a sputum culture showed numerous hemolytic staphylococci (Staph. aureus). Blood cultures made on October 5 and 10 were negative. There was difficulty in grouping the blood prior to transfusion, and it proved to be due to cold agglutinins, which were present in a titer of 1:160 on October 9 and 1:1,280 on October 16. When tested in the warm state the blood was found to be group O. Because of the marked leukocytosis and the appearance of icterus on October 8 and the later discovery of severe anemia, it was assumed that acute hemolytic anemia developed on Octboer 7 or possibly one or two days earlier. Hemoglobinuria was not made out, but the urine of October 8 gave a stronger than usual reaction for albumin. Successive roentgenograms of the chest showed increasing amounts of miliary mottling of both lung fields with some larger irregular areas of density, which varied in location in the different films (fig. 3).

Autopsy (one and one-half hours after death).—Each pleural cavity contained approximately 100 cc. of turbid amber fluid. There were no adhesions. The left lung weighed 600 Gm. and the right 875 Gm. The pleural surfaces of the upper lobes were pale gray to reddish gray. The lower lobes and the dependent portion of the middle lobe of the right lung showed extensive areas of dark bluish purple parenchyma, with only small portions of paler gray and reddish gray tissue in the anterior portions. Showing throughout the pleural surfaces, particularly those of the upper lobes, were numerous small reddish black areas, averaging 2 to 3 mm. in diameter, in many of which there was a small pale yellowish red central nodule. These areas were present throughout the lung and on palpation produced a fine granular feeling. On section the cut surfaces were moist, reddish to crimson and studded with numerous dark, reddish black, somewhat irregular areas, varying from 2 to 5 mm. in diameter, which in a few instances had yellowish nodules similar to those seen in the pleura. Rather large quantities of frothy serosanguineous fluid could be expressed from the surfaces. The bronchi and trachea were lined with dusky, yellowish tan mucosa covered with moderate amounts of thin mucoid material.

Microscopic Examination.—In sections of the lungs many alveoli contained numerous desquamated alveolar lining cells, some of which had vacuolated cytoplasm and the majority of which contained carbon. Some of these cells were binucleate and an occasional one was multinucleate. Practically all the alveoli

containing these cells also contained other mononuclear cells and red blood cells (fig. 4). Other alveoli contained precipitated albumin and desquamated alveolar lining cells. A few alveoli were filled with polymorphonuclear leukocytes, and here numerous cocci were present. An occasional alveolus showed organization. Some bronchioles contained polymorphonuclear leukocytes and cocci, and several showed beginning organization of the intraluminar exudate. There was a peribronchial and perivascular infiltration of plasma cells, some lymphocytes and in places polymorphonuclear cells. Several small arteries contained partially organized thrombi.

The spleen had numerous plasma cells and polymorphonuclear leukocytes in its pulp. In addition, there were myelocytes and occasional focal collections of erythroblasts. The liver showed a rare small area of focal necrosis infiltrated by lymphocytes, plasma cells and polymorphonuclears. In the kidneys some glomeruli contained an increased number of polymorphonuclear cells in the capillaries in foci. Many tubules contained red blood cells and sometimes also a few polymorphonuclear leukocytes. The interstitial tissue was infiltrated in places by plasma cells, some lymphocytes and an occasional large mononuclear cell.

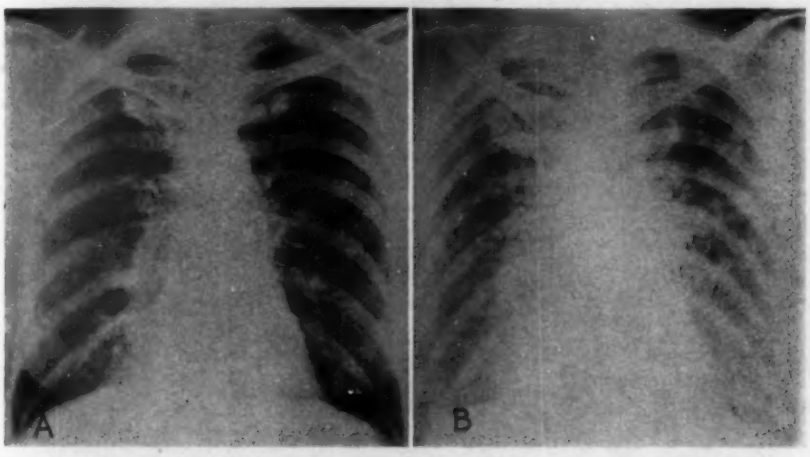

Fig. 3 (case 2).—*A*, roentgenogram of the chest taken on admission. It shows finely mottled densities extending from the right hilus.

B, roentgenogram of the chest taken two weeks after admission, showing miliary soft areas of density throughout both lung fields.

Bacteriologic Study.—In cultures all lobes yielded hemolytic Staph. aureus, occasional colonies of alpha hemolytic streptococci and rare colonies of Hemophilus influenzae. Mice and embryonated eggs inoculated with filtered suspensions of the lungs yielded no recognizable virus.

Case 3 (courtesy of Drs. Henry D. Stebbins, Wayne Hobbs and D. A. Nickerson).—A 53 year old white single woman was well until Oct. 6, 1942, when she began to have symptoms of "grip," with cough, malaise, prostration and a temperature of 102 F. These symptoms continued until she was seen by a physician, on October 12, when she was found to have a temperature of 101 F. and a few rales in the lower lobe of the right lung. She was given sulfadiazine, which she took irregularly until she was sent to the Salem Hospital on October 15. At this time the patient was moderately dyspneic and cyanotic, and showers of rales were heard over the lower lobes of both lungs. The blood sulfadiazine level was

2.4 mg. per 100 cc.; the hemoglobin content, 57 per cent; the red blood cell count, 2,850,000; the white cell count, 28,300, with 77 per cent polymorphonuclears; the icterus index, 18, and the blood nonprotein nitrogen, 42 mg. per 100 cc.

At the hospital, sulfadiazine therapy was continued, 1 Gm. being given every four hours, and the blood level on the day following admission was 12 mg. per 100 cc. The patient was placed in an oxygen tent, but the dyspnea was not relieved. Rales increased until they were heard throughout both lungs. The patient's temperature was 100 F. (rectal) or lower except for a brief rise to 103; the pulse rate was 120 to 140 and the respirations were 40 to 50 per minute most of the time and 60 to 70 during the last day.

On October 16 the urine was noted as being red and as showing albumin (1 plus), a positive reaction for bile and a few white blood cells in the sediment. The blood nonprotein nitrogen stayed between 42 and 48 mg. per 100 cc. A blood culture was negative. Roentgenograms showed diffuse miliary mottling of both lungs throughout (fig. 5). On October 17, the hemoglobin content dropped to 41 per cent, and the white blood cell count rose to 41,500 and on the following

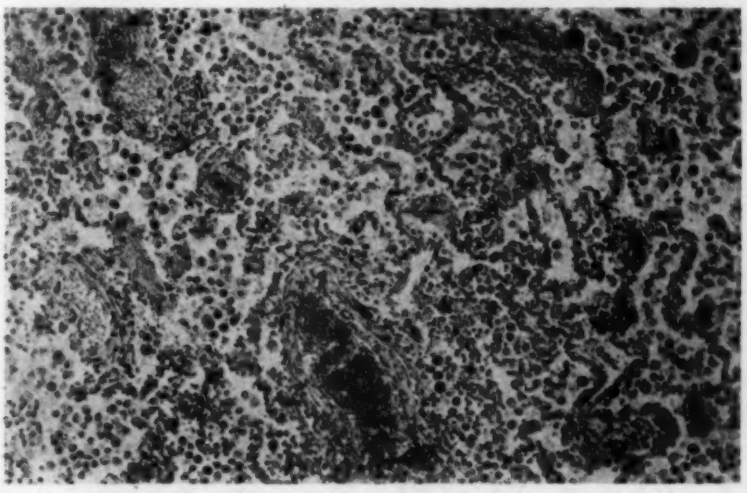

Fig. 4 (case 2).—Alveoli containing an exudate of mononuclear cells. Giemsa's stain; × 100.

day to 57,000, 92 per cent of which were polymorphonuclears. The red blood cell count dropped to 1,200,000 during this time. There was difficulty in typing the blood and in doing cross matchings for transfusions. It was assumed that the patient had a hemolytic type of anemia associated with cold agglutinins, but tests for cold agglutinins were not done. A transfusion was given on October 19. On that day the patient's dyspnea and cyanosis increased; she became markedly delirious and died shortly after the transfusion was completed.

Autopsy (one and one-half hours after death).—The left lung weighed 725 Gm. and the right 660 Gm.; both were grayish and of a rubbery, firm consistence except at the apexes and the bases, which were fairly well aerated. The tissue cut with moderate resistance, revealing a gray surface, from which exuded large amounts of serosanguineous fluid. The cut surfaces were mottled with miliary punctate areas of consolidation spreading out from the hilar regions toward the periphery. The bronchi and trachea contained large amounts of serosanguineous fluid.

Microscopic Examination.—In sections of the lungs some alveoli contained precipitated albumin; others, desquamated alveolar lining cells, monocytes and fibrin, most of which was old and in places was undergoing organization. The lining cells of many alveoli were swollen, and an occasional one was in mitosis. The alveolar walls were infiltrated by plasma cells, monocytes and a few polymorphonuclear leukocytes. Some bronchioles contained mucus and a few polymorphonuclear and mononuclear cells; others were empty. There was a perivascular and peribronchial infiltration of plasma cells. The septums were edematous and were infiltrated by plasma cells and large mononuclear cells. An occasional alveolar capillary was thrombosed.

There was an increased number of plasma cells in the pulp of the spleen. There was an occasional necrotic liver cell invaded by polymorphonuclear leukocytes. Some Kupffer cells contained polymorphonuclear leukocytes and red blood cells. The sinuses of the lymph node contained considerable numbers of mononuclear

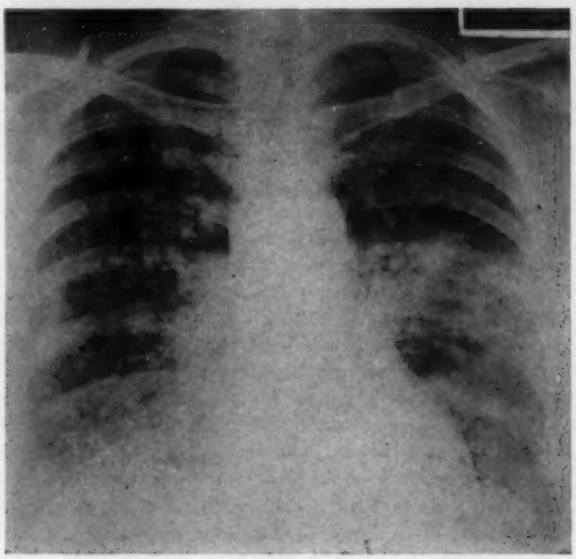

Fig. 5 (case 3).—Roentgen appearance of the lungs on the day before death. There are soft nodular densities in both lungs.

cells, of which some had phagocytosed other cells and some contained carbon. There was an increased number of plasma cells in the lymph cords. The adjacent connective tissue was infiltrated by plasma cells and large mononuclear cells.

Case 4.—A 52 year old white American housewife was in good health until Sept. 25, 1942, when she had aching in the back of her neck and a temperature of 101 F. On the following day she felt "all in" and took to bed. A physician found her temperature to be 103 F. and told her to rest in bed and take the antipyretic drugs which he prescribed. For the next two weeks she complained only of weakness. On October 7 she tried to stay up but felt very dyspneic and had to return to bed. During the next week moderate anorexia developed, and a cough productive of thick yellow sputum, which on two occasions was blood streaked. She also had soreness in the left anterior region of the chest on coughing. She felt cold frequently and perspired at night but had no shaking chills. She was

admitted to the Boston City Hospital on October 14. The past history and the family history were noncontributory. Some of her neighbors had recently been ill with "flu," and her daughter was admitted at the same time with characteristic signs and symptoms of primary atypical pneumonia, which began October 11.

The patient was moderately obese, and on admission she was acutely ill and apprehensive. Her breathing was rapid, short and jerky. Her skin was warm and dry, and her lips and nail beds slightly cyanotic. The pharynx and fauces were red and injected but without exudate. There were moist rales scattered throughout both lungs; in the right lung, posteriorly, they were numerous and coarse. There were no areas of dulness or of abnormal breath or voice sounds. The blood pressure was 138 systolic and 78 diastolic, and the heart rate was rapid. The rest of the physical examination showed nothing remarkable.

The patient was given 4 Gm. of sulfadiazine, followed by 1 Gm. every four hours for three days and every six hours for two more days. She was digitalized over a three day period beginning October 17 and received a maintenance dose thereafter. The rest of the treatment was symptomatic and included oxygen for dyspnea and cyanosis, codeine and expectorants for cough, barbiturates for sedation and stimulants during the latter part of the course.

The temperature was irregular, rising daily to 101 F. at first and later to 102 F., with occasional higher readings. The pulse rate ranged from 100 to 120 per minute during the first five days and thereafter was about 140 except on the last day, when it rose to 160. The respiratory rate rose gradually from 20 to 40 per minute and occasionally was more rapid. Even the slightest exertion was accompanied by marked dyspnea and a sharp increase of the pulse and respiratory rates. The cyanosis had increased markedly by the sixth day in spite of oxygen. The respirations were shallow at first but later became deeper. Coarse rales extended throughout both lungs, and there were also loud, coarse rhonchi from tracheal moisture which the patient could not raise. The patient continued to feel weak and prostrated but remained mentally clear for the first five days; after that she became somewhat disoriented. On the ninth hospital day she became stuporous and later comatose. Her blood pressure fell to 70 systolic and 50 diastolic. She failed to respond to further treatment and died on October 25.

The white blood cell count was 20,100 on entry and thereafter ranged between 15,100 and 21,200, with about 90 per cent polymorphonuclear neutrophils. The hemoglobin content was 70 to 75 per cent and the red blood cell count 6,250,000. On numerous examinations the urine showed good concentration; occasional specimens showed albumin (1 plus), and all had varying numbers of white blood cells and occasional red blood cells in the sediment, and a few sulfadiazine crystals were seen on one occasion. The blood nonprotein nitrogen ranged between 30 and 40 mg. per 100 cc. except for a temporary rise to 67 mg. on October 22. On the latter date the blood carbon dioxide was reported as 68 volumes per cent. Blood sulfadiazine levels rose to 14.4 mg. per 100 cc. on October 17, then gradually dropped to 1.7 mg. by October 23, less than 1 mg. being in the conjugated form in each instance. On October 24 a lumbar puncture showed normal pressure and dynamics and yielded entirely normal fluid. Roentgenograms of the chest, taken on October 17, showed a small patch of mottled density at the base of the left lung, but subsequent ones showed finely mottled infiltration throughout both lung fields (fig. 6). Electrocardiograms, taken on October 19 and 23, showed normal axis, sinoauricular tachycardia, low T_1 and T_2 and inverted T_3 and notched T_4 waves were interpreted as consistent with myocardial disease or digitalis effect.

Repeated cultures of the sputum showed a predominance of alpha hemolytic streptococci, varying numbers of Micrococcus catarrhalis and diphtheroids and

occasional colonies of Staph. aureus. Blood cultures taken before, and on several occasions after, sulfadiazine therapy was started showed no growth. Complement fixation tests for psittacosis and Q fever, made on October 14 and again on October 24, were all negative. The titer of serum cold agglutinins, determined on October 23 and 24, was 1:320.

Autopsy (five hours after death).—Over the outer surface of the right upper arm there were three irregular vesicles, varying from 0.5 to 3.0 cm. in diameter, and on the left upper arm one similar vesicle. These were covered with thin brown skin and contained clear fluid.

The heart weighed 400 Gm. Beneath the endocardium of a region of the left ventricular surface of the interventricular septum were two flame-shaped hemorrhagic areas, 1 by 2.5 cm., with their apexes near the base of the aortic valve.

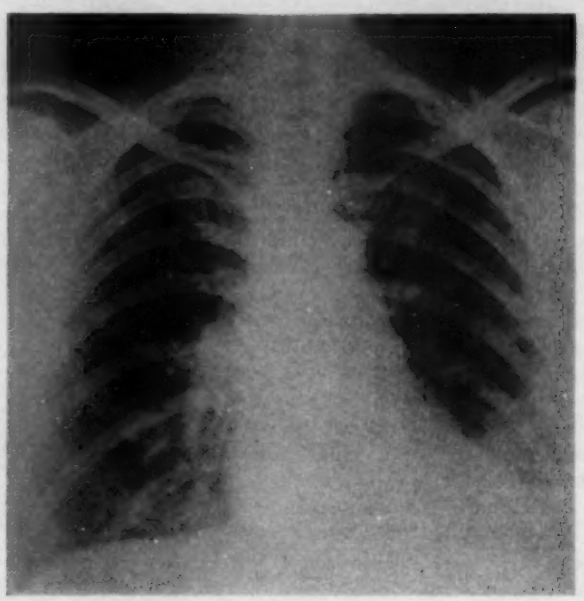

Fig. 6 (case 4).—Roentgen appearance of the lungs one week before death. There are miliary fine soft densities, more in the lower lung fields.

On section these areas were hemorrhagic and extended but a slight distance into the underlying myocardium. The heart was otherwise normal.

The left lung weighed 700 Gm.; the right, 600 Gm. Over the mediastinal surface of the lower lobe of the right lung were seen several hemorrhagic areas, about 1.5 cm. in diameter. The remainder of the surface of the lung was pink, and the lung appeared subcrepitant throughout. On section one of the branches of the pulmonary artery of the upper lobe of the right lung was found occluded by an antemortem thrombus. The lung parenchyma surrounding this vessel was darker and more hemorrhagic. The remainder of the parenchyma was pink and dry. In the left lung there were three localized areas of consolidation, two in the lower lobe and one in the upper lobe, which were wedged shaped, extending from the hilus to the periphery. On section they were raised and firm and were of the same color as the rest of the lung, which was dull pink. The consistence of the remainder of the left lung resembled that of the right.

The brain weighed 1,300 Gm. On coronal section innumerable petechial hemorrhages were found disseminated throughout the cerebral white matter. The hemorrhages were small, averaging about 1 mm. in diameter, although an occasional one measured 2 to 3 mm. in diameter. Few were situated in the cerebral cortex of the central ganglionic masses. In the pons and the cerebellum there were scattered hemorrhages throughout the white matter, but in the medulla few were seen.

Microscopic Examination.—Some alveoli of the upper lobe of the right lung contained albuminous precipitate and red cells; others, polymorphonuclear leukocytes, and still others, varying numbers of polymorphonuclear leukocytes, mononuclear cells and desquamated alveolar lining cells. Some of the latter contained phagocytosed polymorphonuclear leukocytes. There was an occasional multinucleated cell, apparently of alveolar lining cell origin. In some of the alveoli the lining cells were swollen. Some alveoli contained a hyaline membrane. There was a perivascular infiltration of plasma cells and some lymphocytes. A small artery contained an antemortem thrombus with a subendothelial infiltration of monocytes and plasma cells adjacent to it. Many alveoli in sections from the lower lobe of the right lung contained red cells, polymorphonuclear leukocytes and numerous cocci. In some alveoli the lining cells were swollen and desquamated, and vacuolated cells were seen in the lumens. An occasional bronchiole showed beginning organization of the exudate within the lumen. There was a perivascular infiltration of lymphocytes and plasma cells. Many alveoli were essentially normal.

The picture varied greatly from section to section of the left lung. In some the alveoli showed no change. In others they contained numerous red blood cells. Still other alveoli were filled with desquamated lining cells and rare giant cells. One section contained an abscess in which there were masses of cocci. Elsewhere other alveoli contained numerous polymorphonuclear leukocytes and some fibrin. The lining cells of many alveoli were swollen. In still other regions extensive organization of the alveolar exudate was taking place. The bronchioles as a rule were empty, but a few contained mucus, scattered polymorphonuclear leukocytes and some red blood cells. An occasional bronchiole showed organization within its lumen. There was a perivascular infiltration of plasma cells and some lymphocytes and a similar but less striking infiltration of the walls of some bronchioles. A rare blood vessel contained a thrombus.

In the heart there were subendocardial hemorrhages extending into the adjacent myocardium, with necrosis of a few muscle fibers immediately adjacent to the hemorrhages. The spleen was congested, and the pulp contained numerous polymorphonuclear leukocytes, scattered plasma cells and stem cells. A section from the skin showed a vesicle which was filled with albuminous precipitate. The overlying epidermis was necrotic in its deeper portions. The base of the vesicle was made up of connective tissue.

There were widely disseminated lesions in the white matter of the cerebrum and the brain stem. They consisted of small foci of demyelination and proliferation of microglial phagocytes. Some of the lesions were surrounded by extravasated red cells and ring hemorrhages without microglial proliferation (fig. 7). The hemorrhages seemed to be more recent than the proliferative lesions. Many of the lesions were perivenous, but in others a centrally placed blood vessel could not be identified. There were no lymphocytes infiltrating the Virchow-Robin spaces or the leptomeninges. Nerve cells were preserved, even those adjacent to the lesions. No thrombosed vessels were seen.

Bacteriologic Study.—Cultures of the heart's blood yielded no growth. From the upper lobe of the left lung hemolytic Staph. aureus, a streptococcus with alpha

hemolysis and Eberthella coli and Clostridium welchii were isolated. Attempts to isolate a virus from suspensions of the lung were unsuccessful.

CASE 5 (courtesy of Drs. Leroy E. Parkins and Shields Warren).—A 40 year old white married woman was admitted to the Baptist Hospital on Oct. 22, 1942. She first became ill on October 12 with coryza, a raw feeling of the throat and some cough. Her physician found her to be acutely ill, with fever, diffusely injected throat and mucoid nasal discharge. She was given sulfadiazine, 1 Gm. every four hours, and improved temporarily, but her symptoms rapidly returned and became worse. On October 19 a few rales were heard over the lower lobe of the right lung.

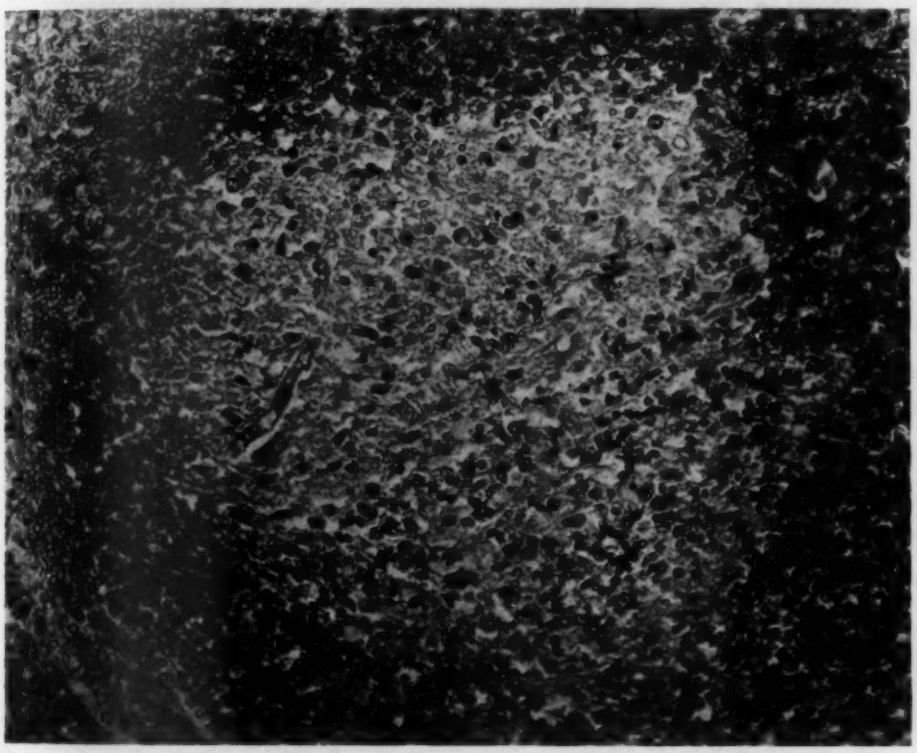

Fig. 7 (case 4).—Brain; perivascular hemorrhages and glial proliferation. Phloxine-methylene blue stain: × 350.

On admission she was extremely ill, with marked dyspnea and some stridor. Her respirations were "moist" and noisy. There was patchy exudate on the tonsillar crypts, as well as a thin gray membrane on the soft palate and the tongue. Throughout both lungs there were showers of rales. The temperature ranged mostly between 102 and 104 F.: the pulse rate was about 120 per minute during the first four days and 140 thereafter, and the respiratory rate rose steadily from 30 to about 60 per minute. The white blood cell count was 12,700, with 82 per cent polymorphonuclear neutrophils on admission; 11,600 on October 25, and 24,000 on October 27. The hemoglobin was 90 per cent and the red blood cell count about 4,500,000 on three occasions. The urine revealed a slight trace of albumin

and no other abnormalities. The blood nonprotein nitrogen was 36 mg. per 100 cc., and the sulfadiazine levels were about 6 mg. per 100 cc. Cultures of mucopurulent sputum showed no significant organisms. A blood culture made on entry was negative. Roentgenograms of the chest on admission showed areas of slightly increased density in the lower lobe of the left lung and some in the lower lobe of the right lung. This density increased in extent and appeared more mottled in successive films until there was miliary mottling involving all of both lung fields (fig. 8).

The administration of sulfadiazine and alkalis was discontinued after three days. The patient was kept in an oxygen tent and given barbiturates for restlessness, codeine for cough, and parenteral fluids. Dyspnea, cyanosis, restlessness, sweating and weakness increased progressively. Bubbling rales were now heard in the chest, and the patient became disoriented and irrational. Later abdominal distention developed, with slight edema of the buttocks, and the patient died on November 1.

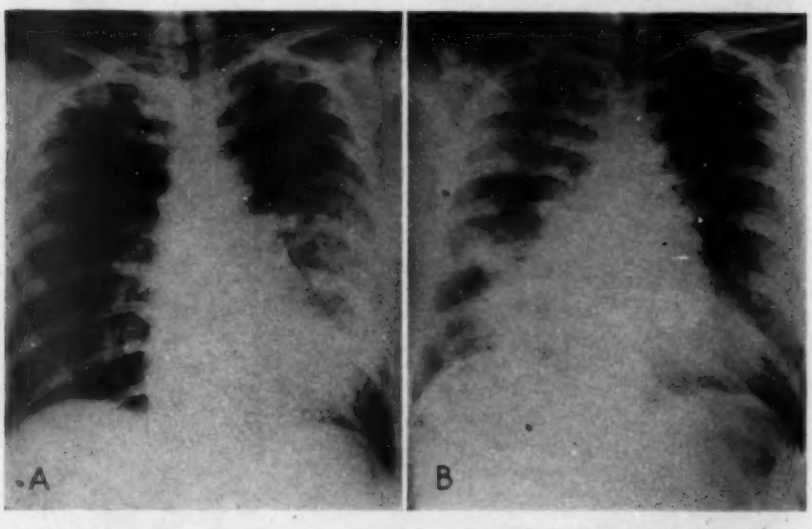

Fig. 8 (case 5).—*A*, roentgenogram taken three days after admission, showing nodular and confluent densities in the left lung field and softer densities extending down and out from the right hilus.

B, roentgenogram taken two days later, showing extension of the lesions to involve most of the right lung and more discrete lesions over the previous area of density on the left.

Autopsy (one and one-half hours after death).—The left lung weighed 500 Gm. and the right 850 Gm.; both were dull deep reddish gray except for the upper lobe of the left lung, which was light gray. The lower lobe of the left lung was much darker than the other lobe. Palpation of the lungs revealed numerous scattered areas, averaging 3 to 4 cm. in diameter, which were much firmer than normal. Sections through the lungs showed moderately firm, dense, red, deeply hyperemic tissue with numerous darker nodular areas throughout all lobes except the left upper lobe, which showed considerable edema. The lung tissue as a whole was firmer than normal, but this change varied from place to place. The firmness was most marked in the lower lobe of the left lung and was generally

present throughout the right lung as well. The bronchi and bronchioles showed slight hyperemia and had a small amount of mucus within the lumens.

Microscopic Examination.—In sections from the lungs some alveoli were filled with edema fluid, some with red blood cells and others with an exudate made up almost entirely of mononuclear cells and desquamated alveolar lining cells (fig. 9*A*). Many of the latter contained phagocytosed material. Other alveoli were filled with polymorphonuclear leukocytes and some with fibrin; this was especially true of the alveoli surrounding bronchioles, which contained a similar exudate. The alveolar lining cells were swollen in many areas, and in these cells mitotic figures were not infrequent (fig. 9*B*). A considerable number of alveoli contained a hyaline membrane. In sections from the lower lobes organization of the alveolar exudate was taking place in many areas. In others the connective tissue of the alveolar walls appeared to be increased. The majority of the bronchioles had little exudate in their lumens, but some did contain numerous polymorphonuclear leukocytes, a few large mononuclears and mucus. There was a rare small focus of necrosis of the lining epithelium. The peribronchiolar and perivascular tissues were infiltrated by plasma cells, lymphocytes, large mononuclears and a few polymorphonuclear cells. A few arterioles contained thrombi.

In the pulp of the spleen there were numerous polymorphonuclear leukocytes and plasma cells and, in addition, a rare myelocyte; also, there was a considerable number of macrophages, many of which contained red blood cells and some of which had phagocytosed various types of leukocytes. Several veins showed a subendothelial infiltration of lymphocytes, plasma cells and an occasional eosinophil. In the liver there were scattered necrotic liver cells; some of these had been completely removed, and the spaces they formerly occupied contained polymorphonuclear leukocytes and occasional large mononuclear cells. The Kupffer cells were prominent; some had phagocytosed red blood cells, others leukocytes. The portal connective tissue was infiltrated by lymphocytes and plasma cells.

Case 6.—This case, too, has been reported in detail elsewhere.[3] The patient was a 24 year old foundry worker whose illness began Dec. 26, 1942 with a shaking chill, high fever, substernal pain and cough, followed in two days by soreness of the mouth and throat and dysphagia. Sulfonamide compounds were then given, without effect, and the patient complained of headache, photophobia, dysuria, bloody sputum and a vesicular rash over the entire body, all of which progressed until he entered the hospital on December 31. He had had similar skin eruptions three times in the previous ten years, and he also frequently had diffuse erythema following ingestion of certain foods. In the hospital he was found to have generalized erythema multiforme exudativum involving the skin, the mucous membranes, the genitals and the conjunctivas and diffuse atypical pneumonia. These all progressed until the patient died on Jan. 18, 1943.

The white blood cell count was 12,250 at first and then dropped to between 3,100 and 6,200, with 91 to 80 per cent polymorphonuclear neutrophils. Urinalyses showed small amounts of albumin, occasional white blood cells and many red blood cells. Roentgenograms showed increasing areas of mottled infiltration of both lungs. Sputum smears showed polymorphonuclear leukocytes and few organisms, and cultures showed only a few alpha hemolytic streptococci at first but later yielded increasing numbers of hemolytic staphylococci (Staph. aureus) and hemolytic streptococci. The blood cultures were all negative. Repeated complement fixation tests for psittacosis were negative. The cold agglutinin titers on January 2, 4, 11, 15 and 18 were $< 1:4$, $< 1:4$, $1:8$, $1:32$ and $< 1:4$, respectively.

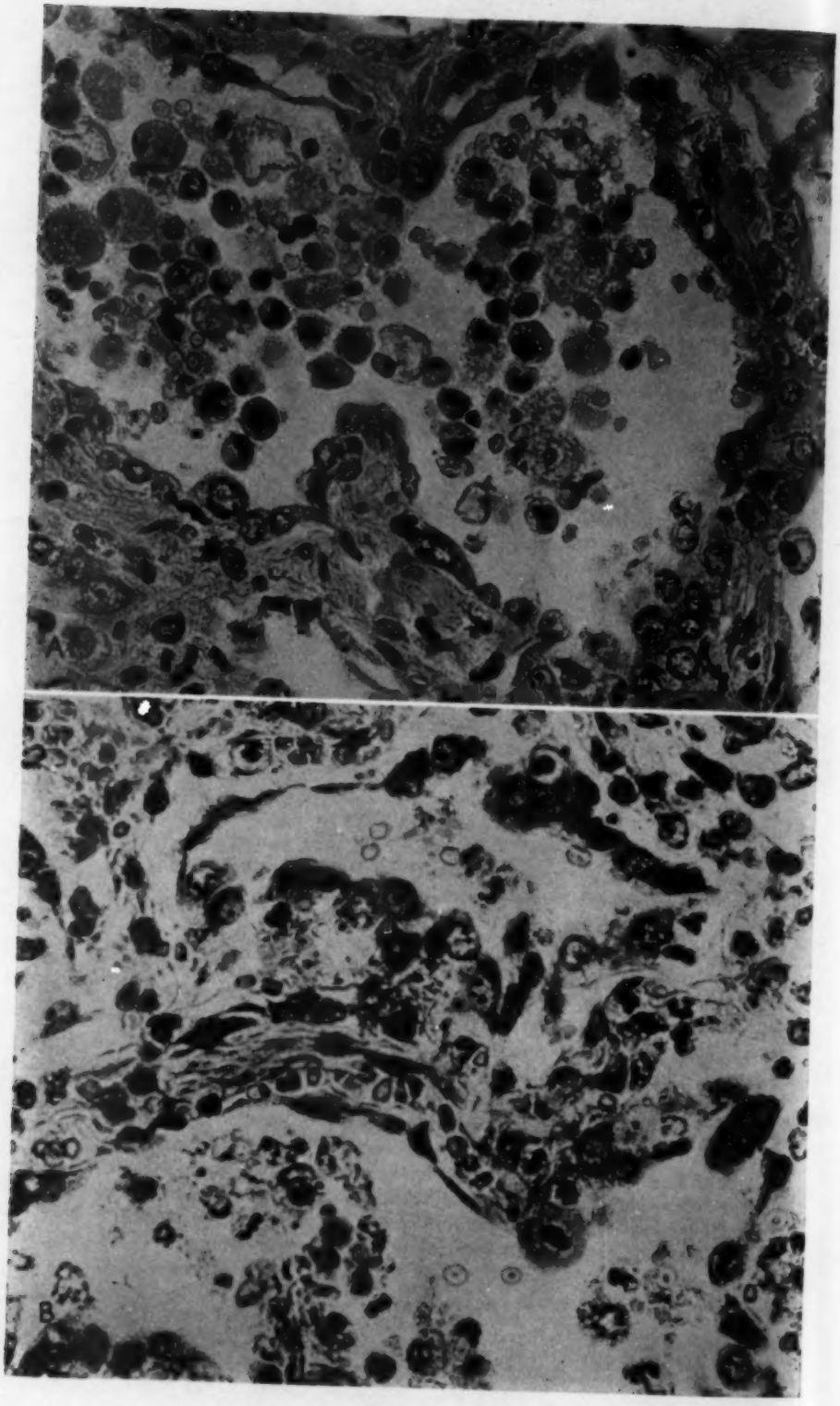

Figure 9

(See legend on opposite page)

Autopsy (fifteen hours after death).—The skin, the oral mucous membrane, the scrotum and the penis showed vesicles and bullae in various stages, some intact, others dried up, encrusted, hemorrhagic or ulcerated. The conjunctivas were markedly injected.

The pleural surfaces were covered with a thin layer of fresh fibrin. The left lung weighed 1,145 Gm.; the right, 1,675 Gm.; both were firm, mostly subcreptitant, with some noncrepitant areas, and their surfaces were deep blue-red and appeared hemorrhagic posteriorly. The cut surfaces oozed a large amount of blood and showed miliary nodules which were yellow-white against a blue to gray-red background and which appeared purulent, but no pus could be expressed. A small amount of pus could be expressed from the smaller bronchioles. The mucosal surfaces of the trachea and bronchi were hyperemic and covered with a slimy dark red exudate containing much blood. The tracheobronchial lymph nodes measured about 2.5 cm. in diameter.

Microscopic Examination.—In sections from several lobes of the lungs the majority of the alveoli contained desquamated alveolar lining cells and large mononuclear cells; the alveolar lining cells were swollen, and varying numbers of mitotic figures were seen. Some alveoli were empty and markedly distended; others contained albuminous precipitate; occasional ones showed hyaline membrane formation; still others contained some polymorphonuclear leukocytes, and many contained fibrin in which there were masses of cocci. The bronchioles contained polymorphonuclear leukocytes, large mononuclear cells and cocci. There was a marked perivascular and peribronchiolar infiltration of plasma cells and some lymphocytes and there were many plasma cells in the alveolar walls and also in their lumens (fig. 10). The septums showed edema. In sections from the upper lobe of the left lung some alveoli contained masses of fibrin, some of which was undergoing organization. Sections from the lower lobe of this lung also showed an area of extensive abscess formation with an exudate of polymorphonuclear leukocytes and many cocci. Adjacent to this area was a focus of gangrene in which numerous cocci and bacilli were present. One bronchiole had lost its epithelium, and its denuded surface was covered with polymorphonuclear leukocytes and fibrin. The pleura was covered with a thin layer of fibrin. The trachea was congested, and the submucosa and the region around the glands was infiltrated by plasma cells.

A section of the skin showed a vesicle containing an albuminous precipitate, some fibrin and a few polymorphonuclear leukocytes. The cells of the inferior surface of the epidermis were necrotic and invaded by polymorphonuclear and large mononuclear cells. The base of the vesicle consisted of the connective tissue of the corium; it was covered with fibrin and showed a perivascular infiltration of lymphocytes and occasional plasma cells. There was a similar infiltration about the coil glands. The cerebral meninges contained a few lymphocytes, large mononuclears and plasma cells. Some of the other organs were infiltrated by similar cells.

Fig. 9 (case 5).—*A*, swollen alveolar lining cells. Alveolar exudate of desquamated alveolar lining cells and large mononuclears. Phloxine–methylene blue stain; × 650.

B, swelling of alveolar lining cells, two of which contain mitotic figures. Giemsa's stain; × 850.

Bacteriologic Study.—Cultures of both upper lobes yielded beta hemolytic streptococci and hemolytic Staph. aureus. Attempts to isolate a virus from vesicle fluid and from a filtered suspension of lung were unsuccessful.

CASE 7.—A 35 year old Jamaica Negro welder entered the Boston City Hospital on Jan. 12, 1943 because of increasing cough and dyspnea. On Dec. 27, 1942 he began to feel tired, lost his appetite and had generalized aches and pains. He worked during the next two days, but on December 30 a severe nonproductive cough developed, associated with some soreness of the chest. On January 1 he raised a small amount of bloody sputum, a culture of which showed no pathogenic organisms. His physician noted diffuse rales in his lungs, diagnosed "virus pneumonia" and prescribed sulfonamide drugs, of which the patient took 1 Gm. every two hours at first and then 1 Gm. every four hours until January 9. During this time his cough and substernal pain increased and he became progressively more dyspneic and cyanotic until he was admitted to the hospital. He had previously been in good health except for attacks of lead poisoning in 1928 and 1929.

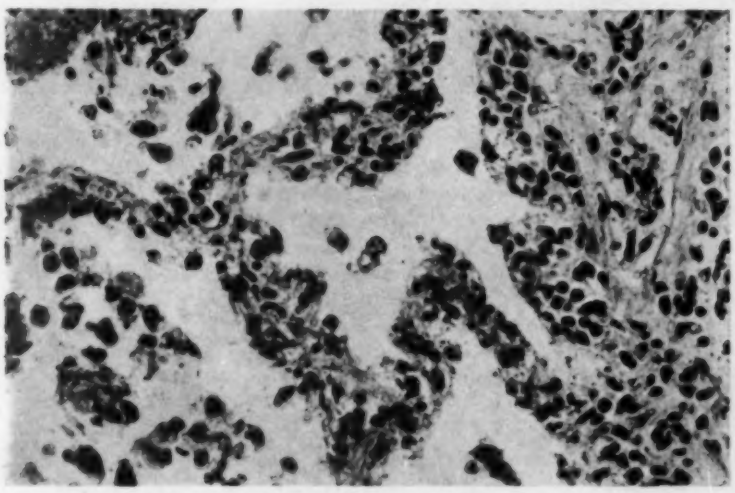

Fig. 10 (case 6).—Alveolar walls infiltrated by plasma cells. Phloxine-methylene blue stain; × 225.

On entry the patient was markedly dyspneic and moderately cyanotic. His respirations were short, grunting, labored and accompanied by some wheezing. His face was flushed and covered with cold sweat. The skin was warm over the trunk and cold over the extremities. The lips and the nail beds were cyanotic. The temperature was 101 F., the pulse rate 138, the respiratory rate 48 and the blood pressure 155 systolic and 95 diastolic. The tonsillar crypts were covered with white exudate, and the vessels of the posterior cavity of the pharynx were injected. There was some dulness of the right upper anterior region of the chest, and some ill defined areas of dulness were present in both lungs posteriorly. Loud musical and crepitant rales were heard throughout both lungs, but there were no definite changes in the breath and voice sounds.

The white blood cell count was 29,800 on admission and 50,000 on the following morning. Practically all of the cells were polymorphonuclears. The urine was normal. A blood culture made on entry showed no growth. The sputum was purulent and slightly blood streaked. Stained smears showed polymorphonuclear

leukocytes, a few epithelial cells, occasional red blood cells and a few gram-positive diplococci and rare short gram-negative rods. Cultures yielded a few alpha streptococci, a few staphylococci and a few H. influenzae. The blood nonprotein nitrogen was 38 mg. per 100 cc. The Hinton test of the blood was negative. The psittacosis complement fixation test of a serum specimen taken January 11 was negative. The cold agglutinin titers, determined on January 11 and 13, were 1:40 and 1:160, respectively.

Sulfapyrazine was administered, 4 Gm., followed by 1 Gm. every four hours to a total of 9 Gm. Fluids were given orally. Oxygen was administered by nasal catheter. The patient's dyspnea and cyanosis, however, increased progressively. On the morning after admission he became excited and suddenly jumped out of bed against the resistance of an attending nurse. He collapsed in the middle of the ward and immediately stopped breathing.

Autopsy (fourteen hours after death).—The apex of the right lung was attached to the chest wall by fresh fibrinous adhesions, which were yellow and easily broken. Elsewhere the surfaces were smooth and glistening. The pericardial cavity contained 40 cc. of thin yellow fluid with a faint bloody tinge. The heart weighed 410 Gm. and showed no abnormalities.

The left lung weighed 740 Gm.; the right, 750 Gm.; both were subcrepitant throughout. On section the cut surfaces oozed a large amount of blood and were rough and finely nodular. These nodules were made up of small pink-white raised areas surrounding bronchioles and measuring 1 to 1.5 mm. in diameter. Otherwise the surface was bright red-gray. Numerous small hemorrhagic areas were present in all lobes except the middle lobe of the right lung. An occasional bronchiole contained yellow purulent material. The mucosa of the bronchi and trachea was pink and velvety. The tracheobronchial lymph nodes were enlarged, measuring 2 cm. in diameter.

The brain showed marked congestion of the meningeal vessels. No other gross abnormalities were made out.

Microscopic Examination.—Sections from the upper and lower lobes of the right lung showed essentially the same picture. Some alveoli contained albuminous precipitate, some polymorphonuclear cells and a moderate amount of fibrin. Some alveoli were distended and lined with a hyaline membrane. In a few places the alveolar lining cells were swollen, and some had desquamated and had surrounded masses of fibrin. The alveolar walls in foci contained fibrin, polymorphonuclear leukocytes and plasma cells. The bronchioles were filled with polymorphonuclear cells. There was a peribronchiolar and perivascular infiltration of plasma cells. The septums were edematous, and the septal lymphatic channels were filled with plasma cells, some polymorphonuclear leukocytes and red blood cells. The great majority of the alveoli of the upper lobe of the left lung contained precipitated albumin. There was a rare small bronchiole filled with polymorphonuclear leukocytes, as were the adjacent alveoli. One large bronchiole had numerous plasma cells and a few lymphocytes infiltrating its wall. In the lower lobe of the left lung there was rather extensive organized pneumonia and beginning organization of the exudate of the bronchioles. The alveoli otherwise were unchanged or contained an albuminous precipitate except in places where the alveoli were lined with cuboid cells, and here their lumens contained desquamated alveolar lining cells, which were often vacuolated. The bronchiolar walls were infiltrated by numerous plasma cells. There was edema of the septums.

The heart had a focal infiltration of a few large mononuclears, plasma cells and mast cells in the interstitial tissue of the myocardium. The spleen contained

an increased number of plasma cells in the pulp and also macrophages, some vacuolated and others containing hemosiderin. The liver showed scattered small areas where the hepatic cells had disappeared, and there were a few lymphocytes, plasma cells and occasional polymorphonuclear leukocytes infiltrating such areas. The testis showed markedly decreased spermatogenesis. In a section from a mediastinal lymph node the sinuses contained many macrophages, some plasma cells and polymorphonuclear leukocytes. There was marked hyperplasia of both the red and the white series of the bone marrow. Sections of the brain revealed no abnormality.

Bacteriologic Study.—Culture of heart's blood showed no growth. Cultures of material from the lower lobes of the lungs yielded a streptococcus with alpha hemolysis.

CASE 8.—A 44 year old mother of two children was known to have had rheumatic heart disease since childhood and during the past eight years had repeated bouts of dyspnea and edema of the ankles, for which she had been taking digitalis regularly. She had been working hard as a waitress when, Oct. 29, 1943, she had a sudden onset of chilliness and malaise, and her temperature rose to 104 F. A severe cough developed, productive of "rusty" sputum, and the patient became dyspneic. A physician diagnosed bilateral lobar pneumonia and prescribed sulfathiazole, 1 Gm. every four hours at first and then 0.5 Gm. every four hours. This therapy was discontinued after two days because of poor urinary output. Fever and symptoms continued and progressed until she was admitted to the Boston City Hospital on November 5.

When the patient entered the hospital, she was moderately dyspneic and stuporous. There were dulness and diminished breath sounds over the lower lobe of the left lung posteriorly, with some wheezing in that area, and showers of crepitant rales throughout both lungs. The heart was moderately enlarged. The sounds were of good quality but grossly irregular, and there were signs of well advanced mitral stenosis. The blood pressure was 110 systolic and 60 diastolic. The abdomen was tympanitic and distended, and there was tenderness in the right upper quadrant, but the liver and the spleen were not felt, and there was no peripheral edema.

Sulfapyrazine was administered, 3 Gm. on admission and 1 Gm. every six hours thereafter. The patient also received a maintenance dose of digitalis, aminophylline for wheezing, and barbiturates and dihydromorphinone hydrochloride ("dilaudid hydrochloride") for sedation and restlessness. Fluids were given by mouth and by hypodermoclysis. Oxygen was given by tent and later was mixed with helium and administered under positive pressure, without relief. The temperature ranged between 102 and 104 F., the pulse rate between 100 and 120 and the respiratory rate between 30 and 45. The musical and coarse rales increased throughout both lungs, but no definite signs of consolidation were made out. The patient became progressively dyspneic and stuporous and died on November 10.

The white blood cell count on entry was 12,400, with 80 per cent polymorphonuclears, and thereafter the counts ranged between 11,400 and 14,200. Several urine specimens ranged in specific gravity from 1.022 to 1.030; all showed albumin (3 plus) and occasional white blood cells. The blood nonprotein nitrogen was 28 mg. per 100 cc. on admission and rose progressively to 56 mg. on November 8 and to 72 mg. on November 10. Daily determinations of sulfapyrazine level showed a progressive rise from 3 to 10 mg. per 100 cc., of which from 0.5 to 4 mg., respectively, were in the conjugated form. Roentgenograms of the lungs, November 6, revealed irregular infiltration of the left lower and the right middle lung field with enlargement of the heart, which showed the typical rheumatic deformity. Some extension of the pulmonary infiltrations was seen in the film of November 9.

An electrocardiogram taken on admission showed auricular fibrillation. Several specimens of sputum were all rusty, showed numerous polymorphonuclears and red blood cells and rare gram-positive extracellular diplococci. Sputum cultures showed alpha hemolytic streptococci at first, but hemolytic staphylococci (Staph. aureus) appeared in great numbers on November 10. Blood cultures, made on entry and again on November 10, showed no growth. The titer of cold agglutinins, November 9, was 1 : 80.

Autopsy (eighteen hours after death).—The right pleural surfaces were smooth and glistening. The left pleural cavity was completely obliterated by old fibrous adhesions. The pericardial cavity contained 200 cc. of clear amber fluid. The heart weighed 550 Gm. The chordae tendineae of the mitral valve were shortened and thickened. The mitral valve was thickened, with marked interadherence of the cusps. Before being opened the valve admitted the tip of the finger only. The aortic valve showed a slight degree of roughening. In the left auricle there was an organizing thrombus.

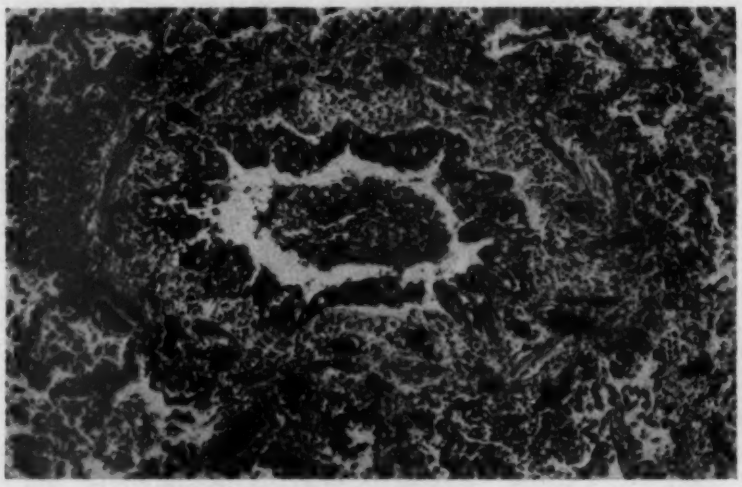

Fig. 11 (case 8).—Lesion of bronchiole similar to that shown in figure 2. The epithelium is intact and increased in thickness. Phloxine–methylene blue stain; × 100.

The left lung weighed 750 Gm.; the right, 950 Gm. The apexes were brown and crepitant, and the bases were congested and edematous. The lower lobe of the right lung had a small amount of pus in the bronchioles. Elsewhere there was no evidence of exudate in the bronchioles, the bronchi or the trachea.

Microscopic Examination.—Some alveoli of the upper lobe of the right lung contained desquamated alveolar lining cells; others, similar cells, monocytes and polymorphonuclear leukocytes. In a few alveoli there were clumps of fibrin surrounded by mononuclear cells. The alveolar lining cells, as a rule, were swollen. The alveolar walls were infiltrated in places by plasma cells and polymorphonuclear leukocytes. The bronchioles contained mucus and a few polymorphonuclear cells. There was a peribronchiolar and perivascular infiltration of a moderate number of plasma cells, some polymorphonuclear leukocytes and an occasional mast cell. The septums were edematous. In the lower lobe of the right lung some alveoli were empty and distended, while others contained an albuminous precipitate,

desquamated alveolar lining cells and large mononuclear cells. The great majority contained numerous mononuclear cells, and in many places a varying number of polymorphonuclear leukocytes as well. A few alveoli contained fibrin surrounded by mononuclear cells. In many alveoli the alveolar lining cells were swollen. The alveolar walls were infiltrated by plasma cells and in foci also by polymorphonuclear leukocytes. The bronchioles contained mucus, some mononuclear cells and polymorphonuclear leukocytes. There was a perivascular and peribronchial infiltration of a moderate number of plasma cells and some polymorphonuclear leukocytes, and the bronchiolar epithelium was thickened (fig. 11). Several blood vessels contained thrombi. A Lee-Brown stain showed reduplication of the capillaries but slight if any thickening of the capillary basement membrane. The lower lobe of the left lung was similar to that of the right lung.

The heart had several rather large scars in the myocardium. The liver showed an occasional necrotic cell invaded by polymorphonuclear leukocytes.

Bacteriologic Study.—Cultures of the heart's blood and of the upper and lower lobes of the right lung showed no growth. A streptococcus with alpha hemolysis was cultured from the lower lobe of the left lung.

SUMMARY OF CLINICAL FINDINGS (TABLE)

Of these 8 cases, 7 occurred in the latter months of 1942, and 1 occurred the following year. Three of the patients were males, aged 17, 24 and 35 years, and the others were females between 40 and 53 years old.

The illness, in these cases, was characteristic of the severe and extensive type of primary atypical ("viral") pneumonia.[1] It began either with general malaise, "grippy" feeling and fever or more abruptly with chills or chilly sensations. These were followed by cough, which was productive of scant mucoid and sometimes blood-streaked sputum and was accompanied by pain or soreness of the anterior part of the chest. The course was characterized by increasing dyspnea and cyanosis, then air hunger and delirium, sometimes by stupor and coma terminally. In 2 cases there was a diffuse bullous type of erythema multiforme and in 2 others exudative pharyngitis. In 1 case there was rheumatic heart disease with mitral stenosis, but the heart was compensated at the time of the onset of pneumonia.

In the lungs the characteristic signs were the increasing numbers of crepitant rales, which were eventually heard throughout; there were only transient areas of dulness posteriorly, with either suppression or increase of breath sounds, but no persistent areas of consolidation. Musical rales or wheezing were heard only occasionally in some cases, and loud rhonchi were heard terminally. There was no improvement from sulfonamide drugs, and oxygen gave only partial, if any, relief in the later stages.

There was slight to marked polymorphonuclear leukocytosis, and in only 2 cases was leukopenia noted during part of the course. The red blood cell counts were normal except in the 2 cases in which acute

Certain Relevant Data in Eight Fatal Cases of Primary Atypical ("Viral") Pneumonia

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Sex and age	Male, 17	Female, 50	Female, 53	Female, 52	Female, 40	Male, 24	Male, 35	Female, 44
Dates:								
Admission	8/26/42	9/30/42	10/15/42	10/14/42	10/22/42	12/31/42	1/12/43	11/6/43
Onset	8/12	9/27	10/6	9/25	10/12	12/36	12/27/42	10/29
Death	9/3	10/18	10/19	10/25	11/1	1/18/43	1/13/43	11/10
White blood cell counts								
(× 1,000)	12.2-17.5	4.7-32.6	28.3-57.0	15.7-21.2	11.6-24.1	12.3-3.1	29.8-50	11.4-14.2
Polymorphonuclears, %	60-85	68-96	77-94	90	82	91-80	92-98	80
Bacteriologic findings* in:								
Blood	0	0	0	0	0	0	0	0
Sputum	Streptococcus with alpha hemolysis; Staph. aureus	Staph. aureus	.....	Streptococcus with alpha hemolysis; Staph. aureus	Streptococcus with alpha hemolysis	Streptococcus with alpha hemolysis; Staph. aureus	Streptococcus with alpha hemolysis; Staph. aureus; H.influenzae	Streptococcus with alpha hemolysis; Staph. aureus
Cardiac blood (autopsy)	0	0	.....	0	.....	0	0	0
Lungs (autopsy)	Staph. aureus	Streptococcus with alpha hemolysis; Staph. aureus; H. influenzae	.....	Staph. aureus; streptococcus with alpha hemolysis; E. coli	.....	Streptococcus with beta hemolysis; Staph. aureus	Streptococcus with alpha hemolysis; Staph. aureus	Streptococcus with alpha hemolysis †
Serologic tests* for:								
Psittacosis, complement fixation titer	1:256	0	.....	0	.....	0	0	.....
Q fever, complement fixation titer	.....	0	.....	0	.....	0	.....	.....
Cold agglutinins	1:640	1:160 to 1:1,280	+ (?) ‡	1:320	.....	<1:4 to 1:32	1:40 to 1:160	1:80
Virus isolated	0	0	.....	0	.....	0	0	.....
Complications	Erythema multiforme exudativum	Hemolytic anemia	Hemolytic anemia	.....	.....	Erythema multiforme exudativum	.....	Mitral stenosis
Sulfonamide therapy, days	9	11	5	5	±18	2	9	2

* A zero indicates that the cultures (or tests) were negative; a blank space, that no cultures (or tests) were made.
† This was from one lobe only, two other lobes yielded no growth.
‡ Tests were not done, but hemolytic anemia and difficulty of cross matchingfor transfusion suggest that cold agglutinins were present.

hemolytic anemia was present.[4] Blood cultures were all negative. Sputum smears showed polymorphonuclear leukocytes and few organisms, and cultures showed predominantly alpha hemolytic streptococci, and most of them later yielded staphylococci. There were also hemolytic streptococci and Hemophilus influenzae in 1 case each. Cold agglutinins were found in moderate or high titer in all of the 6 cases in which serum was tested. A significant titer of complement fixation for psittacosis was found in only 1 of 5 cases, and the tests for Q fever were negative. In every case roentgenologic examination revealed a characteristic soft miliary type of density, which eventually spread to involve most of the lung fields, and in some cases there were more diffuse areas of density that probably represented transient atelectasis.

The duration of the disease from the time of the first symptom was thirteen days in 2 cases, thirty-one days in 1 case and seventeen to twenty-four days in the remaining 5 cases.

SUMMARY OF THE PATHOLOGIC OBSERVATIONS

Grossly, the lungs were enlarged and increased in weight. They presented an appearance of congestion, and there were scattered areas in which crepitus was diminished or absent especially in the posterior and inferior portions. The surfaces overlying the areas of atelectasis were sharply outlined and dark purple-red. The upper and anterior portions were more crepitant, and here the surfaces were more grayish white. There was some scattered fibrinous exudate on the surface in half of the cases. The cut surfaces presented a characteristic miliary nodular appearance, most impressive in the lower lobes, the nodules varying from 1 to 2.5 mm. in diameter, usually appearing grayish white to yellowish against a deep hemorrhagic background. No exudate could be expressed from the nodules; bloody fluid was expressed from the surrounding surface, and only small amounts of mucopurulent materials were expressed from some of the bronchioles. The mucosa of the trachea and the bronchi was generally hyperemic and covered with dark mucus.

The characteristic histologic features, in these cases of primary atypical pneumonia, were the nature of the alveolar exudate, the swelling and proliferation of the alveolar lining cells and the interstitial infiltration.

The alveolar exudate was made up primarily of mononuclear cells. These cells appeared to be of two types—desquamated alveolar lining cells, and large mononuclear cells. The alveolar lining cells were the largest cells. They had a round nucleus and abundant acidophilic cytoplasm, which was sometimes vacuolated. The cells sometimes contained phagocytosed material of various sorts, such as carbon and nuclear

4. Finland, M.; Peterson, O. L.; Allen, H. E.; Samper, B. A., and Barnes, M. W.: J. Clin. Investigation **24**:458, 1945.

débris. Mitotic figures occurred in these cells but were rare. As a rule, the desquamated alveolar cells had a single nucleus, but two or more were occasionally seen. The large mononuclear cells were medium sized, with a round or an indented nucleus. Their cytoplasm was acidophilic and not infrequently contained phagocytosed material. Plasma cells also occurred in some instances. In addition to this cellular exudate, varying amounts of edema fluid, red blood cells and fibrin were present.

The swelling of the alveolar lining cells was a prominent feature. The cytoplasm of such cells tended to be basophilic. Mitotic figures were not infrequent. The shape of these cells was often oval, and their appearance differed markedly from the cuboid type of lining cell seen in other conditions, such as fibrosis of the alveolar walls associated with heart disease, healed tuberculosis, staphylococcic peneumonia or complicating influenza or any other condition which leads to scarring. Necrosis of the alveolar lining cells was not seen; so presumably their swelling, proliferation and desquamation were a reaction to the presence of the causative agent.

The interstitial infiltration was a constant and conspicuous feature. This infiltration occurred in the walls of the bronchioles and around the blood vessels and frequently extended into the walls of the alveoli. The cellular components of this infiltrate were for the most part plasma cells, some of which were immature. Other types of cells present were lymphocytes and mast cells.

The bronchioles, with a rare exception, showed no evidence of injury and contained no exudate except for those in areas where a secondary bacterial infection was present. In these areas the lumens of the bronchioles contained numerous polymorphonuclear leukocytes and often some mucus and, in addition, cocci. In some but not all of the cases in which such secondary infection was present, the alveoli also contained polymorphonuclear leukocytes and sometimes fibrin.

Fibrinous pleuritis of any significant extent occurred only in the presence of secondary bacterial invaders.

Edema of the septums was also a prominent feature, and the septal lymphatic channels often were filled with a cellular exudate made up of plasma cells and various types of leukocytes.

Thrombi were a frequent finding, especially in the smaller arteries and veins. The cause of the formation of such thrombi was not entirely clear, since they were by no means always associated with a contiguous acute inflammatory lesion. The vessel walls showed no visible lesions aside from an occasional subendothelial cellular infiltration. Some of these thrombi had become endothelized and some had undergone partial organization.

Hyaline membranes were found in the alveoli in approximately one half of our cases.

Organization of the alveolar and bronchiolar exudates was found in the majority of the cases but was not extensive.

The only lesions of note occurring in other organs were found in the liver and the brain. There was some necrosis of the hepatic cells, involving only single cells or a few cells; in no case was it extensive. Whether these lesions can be attributed to the etiologic agent of atypical pneumonia, it is impossible to say, since in all our cases the respiratory system was secondarily infected by bacteria to some extent. The lesions of the brain consisted of perivascular hemorrhages with some glial proliferation and were conspicuous in only 1 case.

COMMENT

The pathologic changes observed in our cases correspond with those described by other authors.[2] Longcope [2a] felt that, while the lesions are not unique, they are distinctive and are entirely different from those of pneumococcic pneumonia and lobular pneumonia due to common micro-organisms. The pathologic changes as a whole appear to be more like those of psittacosis [5] than those of any other disease. However, the lesions are not identical in that the alveolar exudate of psittacosis contains considerably more fibrin and red blood cells and interstitial infiltration is by no means so conspicuous a feature as in these cases of primary atypical pneumonia.

The possibility that case 1 was indeed one of psittacosis must be considered because of the high titer of complement-fixing antibodies in the serum. There was no history of the patient's having been exposed to birds in this case, and the pathologic changes noted in the lungs were essentially the same as those in the other cases. Cases 1 and 6 also resembled the 2 fatal cases of "mucosal respiratory syndrome" reported by Stanyon and Warner,[2f] both clinically and pathologically, but in the present cases the cutaneous lesions were more severe and extensive. The significance of the mucocutaneous lesions and their relation to the pneumonia remain obscure. The case with mitral stenosis resembles the one described by Longcope.[2a]

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5. Sturdee, R. L., and Scott, W. M.: A Disease of Parrots Communicable to Man (Psittacosis), Ministry of Health, Reports on Public Health and Medical Subjects no. 61, London, His Majesty's Stationery Office, 1930. Lillie, R. D.: The Pathology of Psittacosis in Man, National Institute of Health Bulletin 161, United States Treasury Department, Public Health Service, 1933. Appelbaum, E., and Ackermann, W.: Ann. Int. Med. **17**:528, 1942. Tanner, F. H.; Covey, G. W.; Everett, H. H., Jr.; Everett, H. H., Sr., and Neely, O. A.: Nebraska M. J. **30**: 386, 1945.

As is true of influenza, secondary bacterial invasion seems to be the rule, but there is a sharp contrast between the changes produced by such bacterial invasion in influenza and those produced in atypical pneumonia. In influenza secondary staphylococcic invasion causes a fatal disease, with death occurring either in a few days or after two weeks or more.[6] In the rapidly fatal cases the most prominent features are edema and hemorrhage, and extensive necrotizing tracheobronchitis and multiple pulmonary abscesses are also seen. No lesions of such extent occurred in our cases of atypical pneumonia. In cases of influenza of longer duration the lungs show extensive fibrosis and cavity formation. In our series 3 patients with atypical pneumonia had evidence of secondary Staph. aureus infection. All 3 lived three weeks or more, but at autopsy no changes resembling those described in cases of influenza were found. In these cases, therefore, the staphylococcic infection was either terminal or mild.

In 4 of our cases streptococci with alpha hemolysis were cultured from the lungs. While the bronchioles contained numerous polymorphonuclear leukocytes and some cocci in places, there was no pyogenic type of reaction in the bronchial walls or in the alveoli. These streptococci, therefore, probably had no relation to the pneumonic process.

The pathologic picture of atypical pneumonia bears a resemblance to that of the experimental pneumonia produced by McCordock and Muckenfuss[7] in rabbits, utilizing vaccine virus. These authors found that a dilute suspension of virus given intratracheally produced a proliferative cellular lesion, which they termed interstitial virus pneumonia.

All attempts to demonstrate inclusion bodies of any type or the L. C. L. bodies[8] of psittacosis in our material, utilizing various staining methods, yielded entirely negative results. Attempts to isolate a virus were made in 6 cases and were all unsuccessful.

GENERAL SUMMARY

The clinical and pathologic observations of 8 cases of primary atypical ("viral") pneumonia have been presented.

Clinically these cases were characterized by increasing symptoms of respiratory embarrassment, diffuse moist rales and transient areas of atelectasis but no definite signs of consolidation of the lungs. Roentgenographically, there was an extensive miliary soft nodular type of density in the lungs. The serum contained cold agglutinins.

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6. Wollenman, O. J., Jr., and Finland, M.: Am. J. Path. **19**:23, 1943. Parker, F., Jr.; Jolliffe, L. S.; Barnes, M. W., and Finland, M.: ibid. **19**:23, 1943.

7. McCordock, R. S., and Muckenfuss, R. S.: Am. J. Path. **9**:221, 1933. Muckenfuss, R. S.; McCordock, H. A., and Harter, J. W.: ibid. **8**:63, 1932.

8. Meyer, K. F., and Eddie, B.: Proc. Soc. Exper. Biol. & Med. **30**:484, 1933.

Two of the cases were complicated by acute hemolytic anemia, 2 others by severe erythema multiforme exudativum and 1 by rheumatic heart disease with mitral stenosis.

The characteristic pathologic changes noted in the lungs grossly were: the congested appearance of the cut surfaces, which were studded with small grayish or dark nodules, and the hyperemic appearance of the mucosa of the trachea and the bronchi. Histologically, there were a mononuclear type of alveolar exudate, an interstitial infiltration predominantly of plasma cells, and swelling and proliferation of the alveolar lining cells. While the bronchioles not infrequently contained polymorphonuclear leukocytes and occasionally some bacteria, their walls were infiltrated by mononuclear cells, and the epithelium was intact. Bacterial infection played a minor role except in 2 cases, in which there was some abscess formation.

Attempts to demonstrate intracellular inclusions or to isolate a virus were unsuccessful.

INTRA-ARTICULAR CHANGES INDUCED IN RABBITS BY INJECTION OF TYPHOID SOMATIC ANTIGEN

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PREVIOUS studies have demonstrated that pathologic changes of certain tissues of rabbits follow the administration of a purified somatic antigen prepared from Salmonella typhosa when this is injected intradermally or intravenously [1a] or when it is used for the induction of the Shwartzman reaction.[2] The present observations concern the effects which this same antigen produces on the articular tissues of the rabbit when it is injected into the joint space or when it is employed as an agent for the production of the Shwartzman reaction in the articular tissues.

MATERIALS AND METHODS

The toxic somatic antigen, prepared from cultures of S. typhosa grown in synthetic liquid medium by a technic involving repeated precipitation with alcohol,[1b] was an aliquot of the material employed previously.[1]

Methods of Administration.—(*a*) Joints: The knee joints of rabbits were shaved and prepared with iodine and alcohol. Antigen, in doses ranging from 0.25 to 0.5 mg., diluted in saline solution, or saline solution alone as a control was injected into the joint space beneath the patella. The total quantity of fluid injected at any one time did not exceed 0.5 cc. Additional injections were made at intervals of from three to seven days for varying periods.

(*b*) Shwartzman Reaction: With rabbits used for the Shwartzman reaction, 1.0 mg. of antigen was injected into a joint space, and 0.5 mg. intradermally into the shaven skin of a flank. Eighteen to twenty-four hours later, these animals received an intravenous injection of 6 to 10 mg. of antigen in 10 cc. of saline solution.

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* Present address: Thorndike Laboratory, Boston City Hospital.

1. Morgan, H. R.: *(a)* Am. J. Path. **19**:135, 1945; (*b*) J. Immunol. **41**:161, 1941.

2. Shwartzman, G.: Proc. Soc. Exper. Biol. & Med. **25**:560, 1928. Morgan.[1b]

Treatment of Tissues.—All specimens except the joint tissues were fixed in Zenker's solution, embedded in paraffin and stained with hematoxylin and eosin. The joint tissues were fixed in formaldehyde solution, decalcified in 5 per cent aqueous nitric acid solution, embedded in celloidin (a concentrated preparation of pyroxylin) and stained with hematoxylin and eosin.

PATHOLOGIC CHANGES INDUCED BY ANTIGEN INJECTED INTO THE JOINT SPACE

The antigen was administered in 0.25 or 0.5 cc. of saline solution (table 1). With several rabbits the same quantity of sterile saline solution was similarly injected into the control joint. Two of the rabbits (119 and 113) had received intravenous injections of somatic antigen (a total of 6.5 mg.) sixty days previously. One (118) had

TABLE 1.—*Data on Experiment in Which Purified Somatic Antigen of Salmonella Typhosa Was Injected Repeatedly into the Knee Joint of the Rabbit*

Rabbit	Total Amount of Antigen Injected, Mg.	Injections	Interval Between Injections, Days	Time of Death After Last Injection	Manner of Death
2	1.5	4	3-4	14 days	Killed with intravenous injection of air
119	1.25	3	3-7	14 days	Killed with intravenous injection of air
49	1.5	4	3-4	28 days	Killed with intravenous injection of air
113	1.25	3	3-7	28 days	Killed with intravenous injection of air
118	1.25	3	3-7	28 days	Killed with intravenous injection of air

received a total of 10 cc. of typhoid vaccine sixty days before the first intra-articular injection of somatic antigen.

Gross Examination.—Twenty-four hours after the first injection of the antigen, the joint was slightly swollen, and the rabbit protected the involved articulation against motion. After seventy-two hours, the joint was moderately swollen, and an effusion was present. These findings increased after repeated injections of antigen and were marked at the time of autopsy, when some of the joints were moderately well fixed in a position of flexion. The joint spaces contained turbid, sticky fluid, and the synovial tissues were hypertrophied and markedly congested. In the animals killed after the longer intervals of time, there was marked atrophy of the periarticular tissues. The control joints appeared normal on gross inspection.

Microscopic Examination.—Stained smears of the intra-articular fluids showed fibrin strands and large numbers of leukocytes with a predominance of polymorphonuclears. The articular fluid of 1 rabbit (118) contained opaque rounded masses of fibrin, ranging up to 2 mm. in diameter and resembling "rice bodies."

The changes occurring in the joint tissues were similar in nature in all animals but were most striking in the animals killed last. The synovial tissues showed marked inflammatory changes characterized by (1) hypertrophy of the lining tissues, (2) diffuse and focal infiltration of both the synovial and subsynovial layers with polymorphonuclears, mononuclears and lymphocytes, (3) congestion and edema and (4) fibroblastic proliferation in the subsynovial layers, especially at the perichondrial margins (fig. 1 *A*). The cartilage showed areas of partial and complete necrosis. In such areas, many empty lacunar spaces were seen, and the matrix appeared fibrillated and uneven. The hypertrophy and clustering of cartilage cells suggested regenerative activity (fig. 1 *B*).

There did not appear to be any consistent or significant differences between the changes elicited in the joints of rabbits that had received intravenous injections of typhoid somatic antigen or typhoid vaccine previously and those resulting from intra-articular injections of antigen in normal animals. The control joints either were normal or showed only minimal inflammatory changes.

Sections of the heart, the lung, the liver, the spleen and the kidney showed no significant changes. Any lesions of these tissues which may have occurred in rabbits 119 and 113 following the previous intravenous injections of somatic antigen apparently had disappeared.

PATHOLOGIC CHANGES FOLLOWING THE PRODUCTION OF THE SHWARTZMAN PHENOMENON IN THE KNEE JOINT AND SKIN

The animals in which intra-articular Shwartzman reactions were induced were killed at varying intervals of time as noted in table 2.

Gross Examination.—In the animals that died before there was gross evidence of the Shwartzman phenomenon, i. e., less than two hours after the injection, marked congestion of the viscera, especially of the liver, was observed. The area of skin into which the injection had been made twenty-four hours earlier was inflamed and edematous. An increased amount of turbid and frequently blood-stained fluid was found in the articular spaces of the swollen joints. The synovial tissues of these joints were reddened and edematous.

With the appearance of the Shwartzman reaction, the prepared area of skin became dark red because of extensive extravasation of blood. The periarticular swelling increased, and the skin overlying the medial and lateral portions of the joint showed a dark purplish coloration due to the underlying hemorrhage. In animals 4 and 48, killed at eight hours, the articular tissues were edematous and hemorrhagic, and the involved joints contained an excess of turbid fluid. There were hemorrhages in the walls of the large intestine, and the kidneys had a mottled appearance.

In aimals dying after long intervals (five days) the site of the cutaneous Shwartzman reaction had become blackened, shrunken and depressed. The skin over the involved knee joint showed similar changes. The joint spaces contained purulent exudate, and the reddened synovial tissues extended over the margins of the patellar surface of

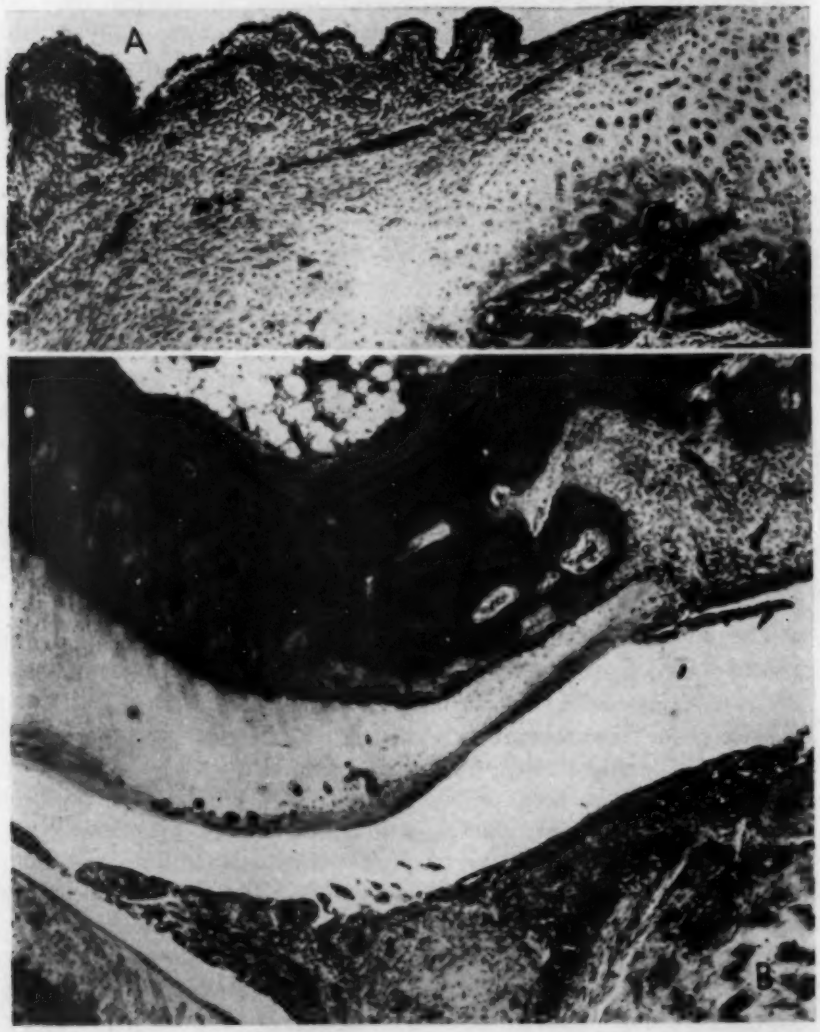

Fig. 1.—*A*, proliferative and inflammatory changes occurring in the synovial and subsynovial tissues at the perichondrial border of the articular surface of a femur. (Rabbit 49, table 1, killed twenty-eight days after the last intra-articular injection of antigen.)

B shows, in addition to the inflammatory changes of the synovial tissues, the necrosis of articular cartilage that was observed in areas. Clustering of cartilage cells is also apparent. (Rabbit 118, table 1, killed twenty-eight days after the last intra-articular injection of antigen.)

the femur. In rabbit 124, killed at nine days, the knee joint showed even more extensive proliferation of the synovial tissues. At twenty-eight days (rabbit 133) the knee joints were markedly swollen and the adjacent muscles atrophied. The articular spaces contained an excess of turbid fluid. The greatly thickened synovial tissue had overgrown a thinned and atrophied articular cartilage.

Microscopic Examination.—The synovial fluids obtained from the knee joints at various intervals following the induction of the Shwartzman reaction showed numerous polymorphonuclears, mononuclear leukocytes and erythrocytes. In the animals killed after periods of one-half hour to twenty-four hours, the tissues showed a reaction characterized by capillary hemorrhage and thrombosis of the blood vessels.

TABLE 2.—*Data on Experiment in Which the Shwartzman Phenomenon Was Produced in the Knee Joint of the Rabbit with Purified Somatic Antigen of Salmonella Typhosa*

Rabbit	Severity of Shwartzman Reaction		Time of Death After Intravenous Injection	Manner of Death
	Knee Joint	Skin		
125	0	0	½ hr.	Died
127	0	0	½ hr.	Died
130	0	0	½ hr.	Died
132	+	+	2 hr.	Died
4	++	++	8 hr.	Killed with intravenous injection of air
48	+++	+++	8 hr.	Killed with intravenous injection of air
128	+++	++	24 hr.	Died
134	++++	++++	5 days	Died
124	++++	++++	9 days	Killed with intravenous injection of air
123	++	++	18 days	Killed with intravenous injection of air
133	++++	++++	27 days	Killed with intravenous injection of air
126	(Control—no intravenous injection)		21 hr.	Killed with intravenous injection of air

The synovial membranes were edematous and congested with a heavy diffuse leukocytic infiltration in which polymorphonuclear leukocytes predominated (fig. 2*A*). These changes became more marked with the passage of time (fig. 2*B*).

As the intervals following the induction of the Shwartzman reaction lengthened, the process within the joint was enhanced by acute and subacute inflammation. The synovial and subsynovial tissues showed focal areas of necrosis on which fibrin was deposited. The upper third or half of the articular cartilage showed loss of cell structure, and the matrix stained poorly. The early proliferative changes of the fibroblasts observed in the synovial and subsynovial tissues at the perichondrial margins and elsewhere were striking. Some blood vessels showed organizing thrombi. In rabbit 133, killed twenty-seven days after the production of the Shwartzman reaction, there was deformity of the articulation, caused by degeneration of the articular cartilage, inflammation, and fibrosis of the capsular tissues, and productive growth of

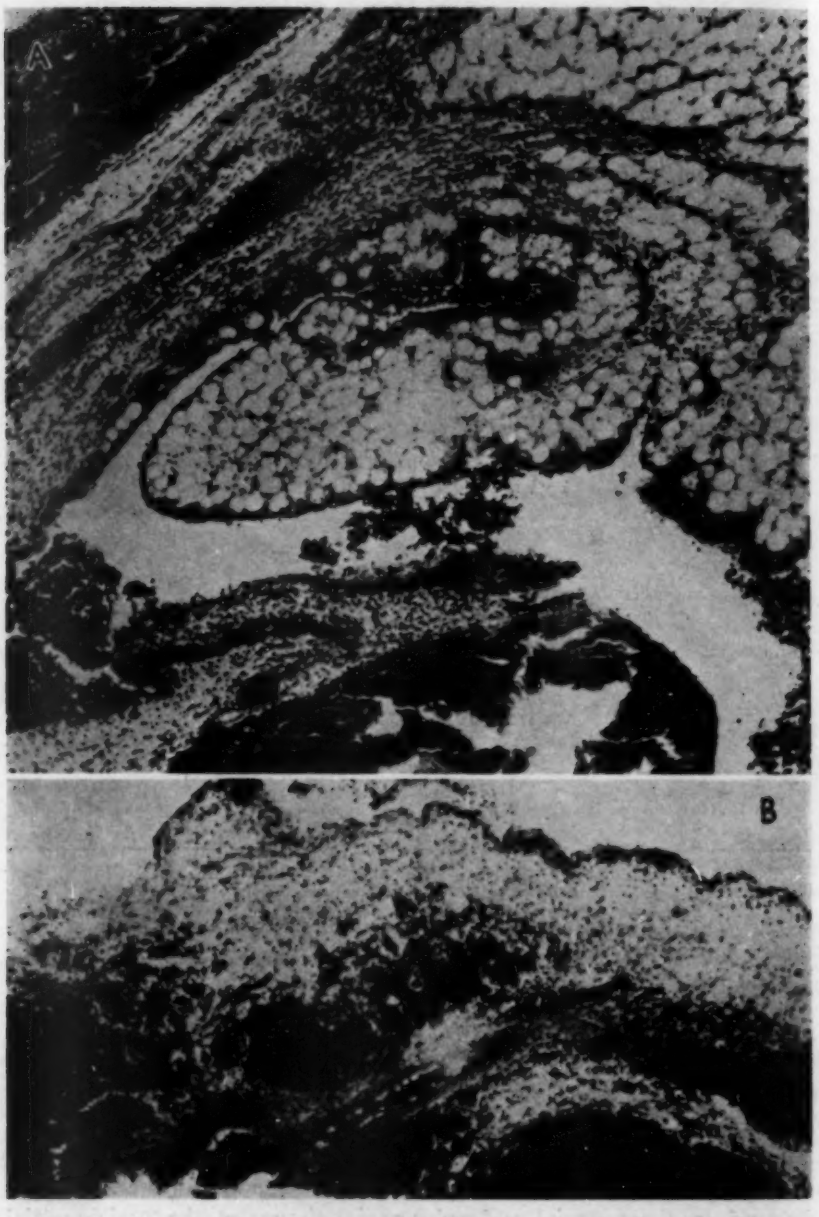

Fig. 2.—*A*, congestion of blood vessels with early extravasation of erythrocytes, which have penetrated into the synovial and subsynovial tissues, and pronounced early leukocytic infiltration of the tissues and the joint space. (Rabbit 130, table 2, which died thirty minutes after the intravenous injection of antigen.)

B, congestion, widespread hemorrhage and thrombosis of blood vessels of the synovial and subsynovial tissues. (Rabbit 48, table 2, killed eight hours after the intravenous injection of antigen. A typical Shwartzman reaction was apparent in the knees and the skin.)

cartilage at the perichondrial margin (fig. 3 *B*). The synovial tissues showed acute and chronic inflammatory changes (fig. 3 *A*) with extensive leukocytic and lymphocytic infiltration. These changes indicated pronounced and continuing injury of all articular tissues.

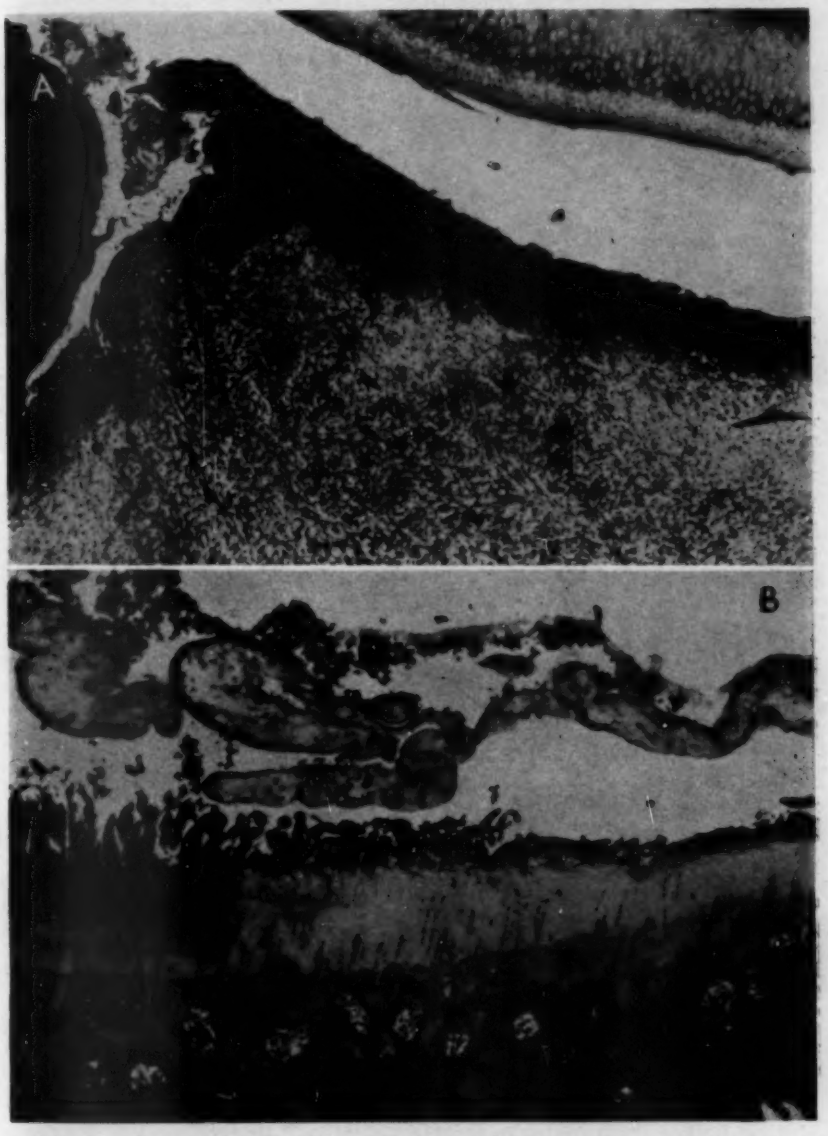

Fig. 3.—*A* shows the synovial and subsynovial tissues extremely thickened and granulation tissue replacing the surface exudate and necrotic tissue twenty-seven days after the production of the Shwartzman reaction. (Rabbit 133, table 2).

B, marked degeneration of the articular cartilage of a femur and a tibia. (Rabbit 133, table 2, killed twenty-seven days after the production of the Shwartzman reaction.)

The area of skin in which the Shwartzman reaction was produced was characterized soon after the onset of the reaction by acute inflammatory changes, hemorrhage, and occasional thrombosis of blood and lymphatic vessels (fig. 4 *A* and *B*). By the end of five days the cutaneous lesion had taken on the character of an eschar with old hemorrhage, necrotic collagen fibers and masses of leukocytes. Later this eschar became sharply demarcated and began to separate from the adjacent

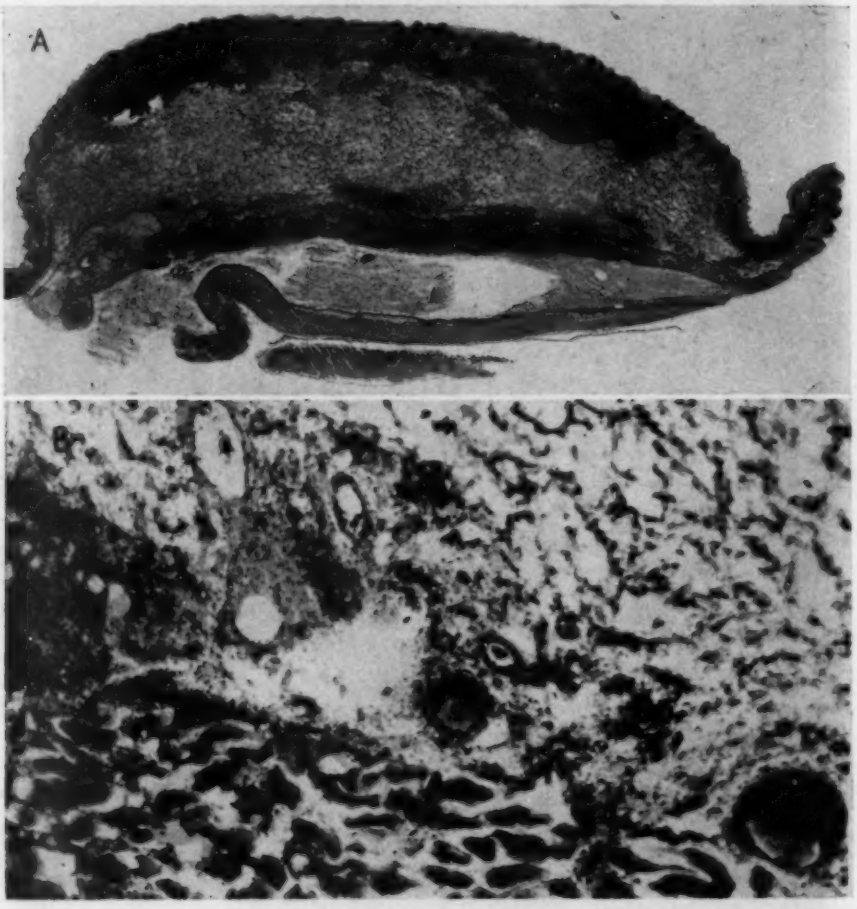

Fig. 4.—*A*, low power photomicrograph showing marked congestion and edema and early hemorrhage in the prepared area of skin eight hours after the intravenous injection of antigen. (Rabbit 48, table 2.)

B, higher magnification of an area of the lesion shown in *A*, revealing edema, leukocytic infiltration, congestion and early thrombosis of vessels.

viable tissues through the formation of granulation tissue (fig. 5). By the eighteenth day this area had either sloughed off or become a sharply circumscribed abscess with a thick fibrotic wall.

The changes observed in the cardiac, pulmonary, hepatic, renal, adrenal and splenic tissues of these animals were similar to those observed following repeated intravenous injections of antigen [18] with certain exceptions. The heart muscle frequently showed more extensive areas of necrosis. These changes were more marked in the right ventricle, which showed extensive calcification in some animals killed after

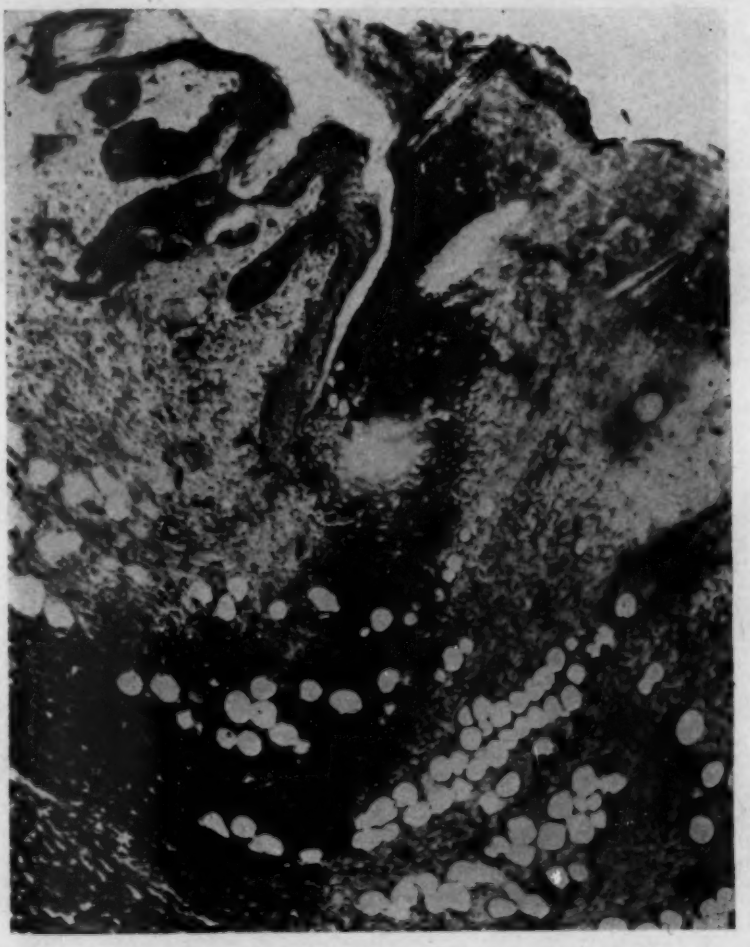

Fig. 5.—A line of separation is seen between the necrotic dermal tissues on the right and the viable tissues on the left. The area of the Shwartzman reaction consists of a necrotic plaque or eschar. (Rabbit 124, table 2, killed nine days after the onset of the reaction.)

intervals of more than a week (fig. 6 *A*). The lungs showed the areas of hemorrhage and the thrombi in blood vessels (fig. 6 *B* and *C*) which were noted in the earlier experiments,[18] but the changes in the blood vessels were more outstanding.

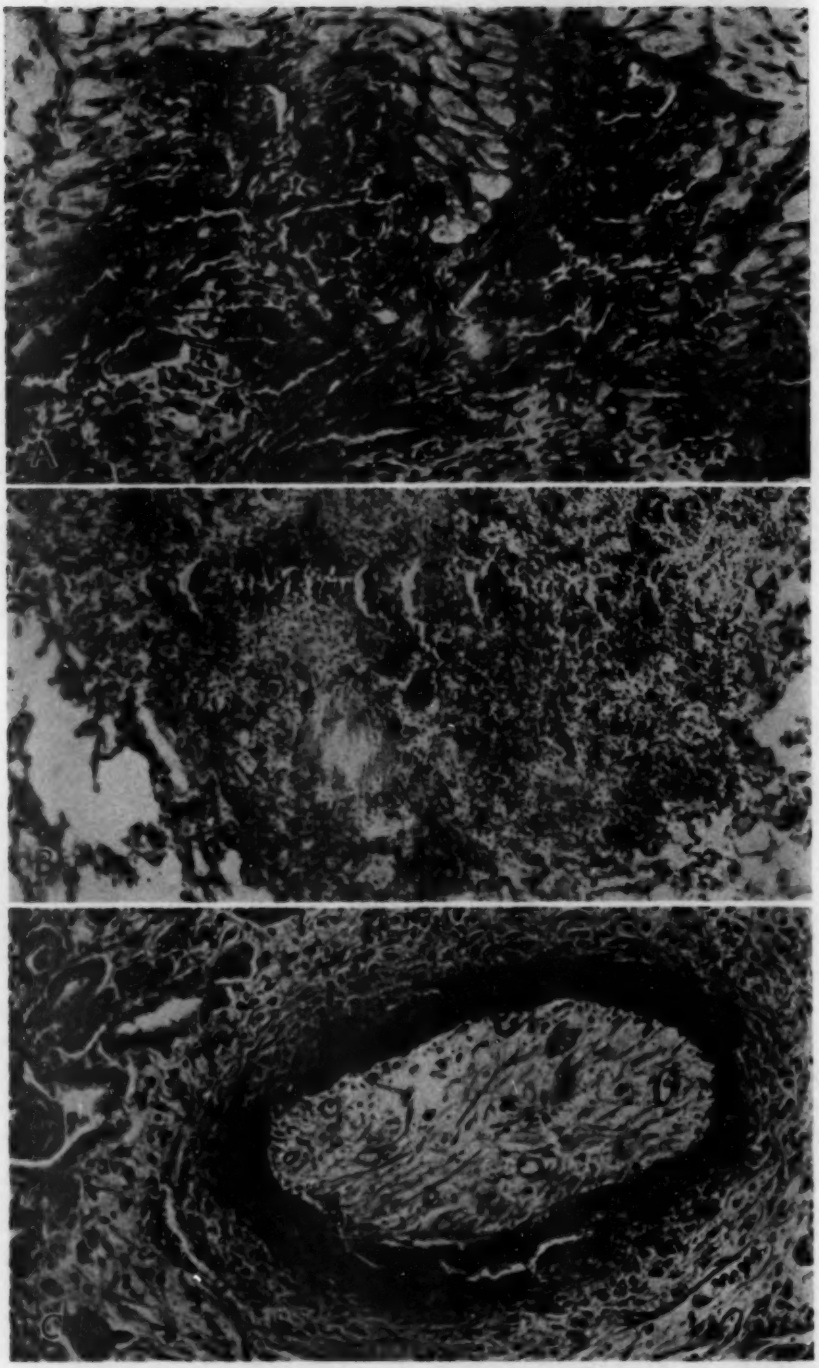

Fig. 6.—*A*, degeneration, fibrosis and early calcification of the myocardium of the right ventricular wall. Near the top of the reproduction are bundles of swollen and vacuolated but viable muscle fibers. (Rabbit 124, table 2, killed nine days after intravenous injection of antigen.)

B, an area of old hemorrhage and fibrosis in the lung. This lesion appeared to have been caused by an injury of blood vessels resulting in thrombosis.

C, higher magnification of an area of lung similar to that shown in *A*. The wall of the pulmonary artery is necrotic and partially calcified. The lumen is occluded by an organizing thrombus. (Rabbit 124, table 2.)

In rabbit 126, which was killed twenty hours after it had received an injection of 1 mg. of somatic antigen into each knee joint, only the adrenal among other body tissues showed changes. There was an infiltration with polymorphonuclear leukocytes, which in some instances had invaded the cortical cells. This reaction was similar to that seen in the animals which had received the intravenous, eliciting injection of antigen. The cardiac, pulmonary, hepatic and renal tissues of this animal showed no significant changes. The fact that lesions were observed only in the adrenal tissues suggests that only small amounts of the somatic antigen injected into the knee joints may have gained access to the blood stream.

COMMENT

The results described indicate that the toxic somatic antigen derived from S. typhosa produced marked changes in the articular tissues when injected directly into the joint space. The intravenous injection of typhoid vaccine or somatic antigen made some days previous to the intra-articular injection of somatic antigen had no effect on the type of changes observed in the tissues of the joint. This suggests that the presence of circulating typhoid antibody does not modify the reaction of joint tissues damaged by this toxic antigen. This effect is similar in nature to the destructive action of the antigen on cardiac, hepatic and adrenal tissue and on bone marrow following its intravenous administration.[18] The latter tissues showed little or no visible change following intra-articular injection of the antigen, suggesting that the antigen did not pass readily from the joint space into the circulation. This observation is in keeping with the previous demonstration of the ability of the antigen to induce rapid inflammatory fixation *in situ*.[18] However, the changes occurring in the adrenal gland of rabbit 126 suggested that minute amounts of the somatic antigen may have entered the circulation from the joints.

In previous studies [18] the intradermal injection of the antigen induced a prompt and severe acute inflammatory reaction. The ensuing lesion soon became encased in a wall of dense connective tissue, and complete healing followed.[18] In sharp contrast, in the present experiments the intra-articular injection of the antigen elicited an acute inflammatory reaction which subsequently became chronic with resulting hypertrophy of the synovial membrane, degeneration of cartilage and gross deformity of the joint. The intensity and the progression of the articular as compared with the cutaneous reaction would seem to indicate that the articular tissues are more vulnerable to this toxic agent.

When used for inducing the Shwartzman reaction in the skin and the joint tissues, S. typhosa antigen caused, in addition to violent acute inflammation, a pronounced vascular injury that resulted in hemorrhage and thrombosis of blood vessels of the adjacent tissues. These changes

were similar to those observed by Moritz and Morley.[3] Again it was observed that the articular lesion was much more persistent than was the dermal lesion. The latter reaction passed through a typical self-limited course with eschar production and prompt segregation whereas the affected joints showed evidence of continued inflammation and proliferation of granulation tissue for as long as twenty-seven days after the Shwartzman reaction had occurred.

The changes observed in the cardiac and pulmonary tissues of these animals may have been due in part to injury resulting from a generalized Shwartzman reaction, for evidence was noted (rabbit 126) that small amounts of the somatic antigen may have entered the circulation after the preparatory intra-articular injections. This material could have acted as a preparatory dose for the subsequent intravenous injection.

SUMMARY

Typhoid somatic antigen when repeatedly injected into the knee joints of rabbits produces acute arthritis, which gradually becomes chronic, leading to hypertrophy of the synovial tissues, with focal accumulations of lymphoid cells in the subsynovial layers, accompanied by destruction of the joint cartilage. No differences were noted between the lesions produced in normal rabbits and those in rabbits that had received previous intravenous injections of typhoid antigens.

When the Shwartzman reaction is induced in the skin and the knee joint, there is extensive vascular injury, resulting in hemorrhage, thrombosis and tissue necrosis. Such lesions undergo repair—in the skin by formation and separation of an eschar, in the joints by productive inflammation that may remain active for twenty-seven days or longer. These animals may also have areas of necrosis and thrombosis of blood vessels in the heart, the lungs and the liver.

3. Moritz, A. R., and Morley, J. D.: Proc. Soc. Exper. Biol. & Med. **29**:321, 1931.

FIBROSIS UTERI

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LOS ANGELES

THE CRITERIA for the diagnosis of fibrosis uteri have never been clearly defined from either a clinical or a pathologic standpoint. In no textbook, not even in those devoted solely to gynecologic disorders, is an adequate description of the disease given. As a result, some physicians, both clinicians and pathologists, deny the existence of the entity; others accept it with little interest as offering an explanation in rare or isolated instances of menorrhagia; still others, unfortunately, employ the term in a loose manner to label the condition of a uterus which when removed contains no lesion ordinarily acceptable as affording an explanation for the patient's symptoms. On the other hand, the surgeon who has made a preoperative diagnosis of uterine myoma or adenomyosis may encounter at operation only a symmetric, slightly enlarged uterus which when palpated in situ reveals uniformly and not greatly increased consistency. He hesitates to remove an organ showing so little gross pathologic change and may resort to a suspension operation or a similar ineffectual procedure without benefit to the patient.

The slightly enlarged, firm uterus which gives rise to menorrhagia has aroused interest for fifty years or more. A number of papers were written on the subject in the early 1900's. Since then interest has waned. Further knowledge of the endometrial cycle has shown the cases of so-called endometritis to be merely instances of normal secretory activity of the endometrium, but the changes in the myometrium and their possible causation of menorrhagia are still poorly understood.

Scanzoni,[1] in 1863, described these changes and considered them a result of chronic metritis, a conception supported by Bell,[2] von Lorentz [3] and Goodall.[4] Findlay [5] did not agree as to an inflammatory causation;

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1. Scanzoni, F. W.: Die chronische Metritis, Vienna, L. W. Seidel u. Sohn, 1863, pp. 1-52.

2. Bell, W. B.: The Principles of Gynaecology, New York, William Wood & Company, 1917, p. 265.

3. von Lorentz: Arch. f. Gynäk. **70**:309, 1903.

4. Goodall, J. R.; Altimas, G. T., and Ayre, J. E.: J. Obst. & Gynaec. Brit. Emp. **49**:18, 1942.

5. Findlay, P.: Am. J. Obst. **52**:71, 1905.

he also discarded the possibility that arteriosclerosis was responsible. He considered the changes to be due to an increase of fibrous connective tissue following prolonged chronic passive congestion. Berkley and Bonney [6] considered the lesion to be fibrosis without any inflammatory basis, in most cases, Novak [7] expressed the belief that in some cases it results from chronic metritis and that in others it represents chronic subinvolution. In 1907 Shaw [8] wrote his thesis "The Pathology of Chronic Metritis." He made Van Gieson-stained sections in a series of cases and measured them carefully. He concluded that the uterus was enlarged by a proportionate hypertrophy of the fibrous and muscular elements. He also divided his cases into four groups depending on the distribution of the connective tissue. In group 1 the connective tissue occurs in densely staining strands between muscle bundles. In group 2 these dense strands also extend between the muscle cells. In group 3 the connective tissue between the muscle bundles is arranged in a loose meshwork, and in group 4 a similar loose meshwork is found between the muscle cells. He does not elucidate this classification further.

In 1944 Williams and Kinney [9] studied a group of 10 cases. They found no fibrosis but instead hypertrophy of the muscle fibers and so proposed the term "myometrial hypertrophy." All their patients were multiparas, and all but 1 patient, 25 years of age, were between 37 and 51 years of age.

Curtis [10] in a recent paper has proposed the term "diffuse hypertrophy of the uterus" to include all the uteri showing moderate diffuse enlargement regardless of the etiologic factors or the pathologic observations.

The confusion of terminology has continued, and the diagnosis has often been made only when no other explanation of the patient's symptoms was found.

The present study was made in an attempt to determine whether the disease is a clinical entity or not and to define the pathologic criteria for making the diagnosis.

MATERIALS AND METHODS

Fresh uteri received from the surgery division were inspected, measured and opened along the middle of the anterior surface. The width and the consistency of the myometrium and the endometrium were noted. The organ was then halved by continuing the incision, and a block of tissue was removed to represent the full width of the uterus in the center of the posterior wall, an area arbitrarily chosen for

6. Berkley, C., and Bonney, V.: A Guide to Gynecology in General Practice, London, Oxford Medical Publications, 1915, pp. 190-191.

7. Novak, E.: Gynecological and Obstetrical Pathology, Philadelphia, W. B. Saunders Company, 1940, p. 159.

8. Shaw, W. F.: J. Obst. & Gynaec. Brit. Emp. **11**:124, 1907.

9. Williams, J. T., and Kinney, T. D.: Am. J. Obst. & Gynec. **47**:380, 1944.

10. Curtis, A. H.: Am. J. Obst. & Gynec. **50**:748, 1945.

purposes of uniformity. The block was fixed in Zenker's solution, to which glacial acetic acid has been added to a concentration of 5 per cent just before use, and stained with Mallory's aniline blue or Masson's trichrome stain. The organ was then sliced by parallel incisions at 5 mm. intervals to demonstrate any additional lesions.

The four layers of the myometrium were identified and marked off on the slide with india ink and wax pencil. These four layers named from within outward are: the submucous, the vascular, the supravascular and the subserous. The dividing lines between them are indistinct because of the interlacing of the muscle bundles, but for the purposes of this study they were delimited as follows: (*a*) The vascular layer, comprising the central two fifths to one half of the myometrium, was identified by its large vessels as seen with an inverted ocular or 32 mm. objective. Its musculature is relatively compact. (*b*) The submucous layer lies between the vascular layer and the endometrium. It is traversed by vessels which run more or less perpendicular to the endometrium. Its musculature is more compact than that of any other layer. Its width is roughly 25 per cent of the myometrium. (*c*) The supravascular layer and (*d*) the subserous layer lie external to the vascular layer and together represent approximately 25 per cent of the width of the myometrium. The subserous layer is very thin and composed of two longitudinal layers separated by an oblique one but the whole very compactly arranged and thus easily distinguished from the supravascular layer, in which the small muscle bundles are loosely arranged in a bulky supporting connective tissue.

The width of each layer was measured with an ocular micrometer, a 4 mm. objective being used, and the fibrous connective tissue content of each layer was measured in the same manner. In the process of measurement all tissue staining blue with Mallory's stain or green with Masson's was considered fibrous connective tissue, and the artefactural spaces within this tissue were measured as though the fibrous tissue were solid. Since the same method of measurement was used in both the cases of fibrosis and the controls, this error is not significant in a comparison of the two; however, it does magnify the apparent fibrous tissue content of all the uteri studied.

Measurement of individual muscle fibers and counting of muscle fibers in a given area were also done in repetition of the work of Williams and Kinney.[9]

In this manner 27 uteri with fibrosis uteri and 28 control uteri were studied. The control group consisted of autopsy and surgical specimens which were normal or contained leiomyomas, adenomyosis or endometrial polyps and which came from patients whose age distribution was the same as that of the patients with fibrosis.

RESULTS OF STUDY

Incidence.—The 27 cases of fibrosis uteri were found in a period of nine months, during which a total of 245 uteri were received for examination, an incidence of 1 in 9, or 11 per cent.

Twenty-six of the patients were Caucasian; one was Negro.

Sixteen were multiparous and 5 primiparous, and 1 was nulliparous. In 5 cases the parous state was not noted.

The youngest patient was 31 years of age; the oldest, 52. The average age was 41 years. Eleven patients were in the fourth decade, 13 in the fifth and 3 in the sixth.

Menstrual History.—The age of onset of menstruation was not remarkable, nor was any correlation found between the early menstrual history and the later development of fibrosis.

All patients but 2 complained of profuse menorrhagia. The exceptions were, first, a 40 year old nullipara operated on for appendicitis, who had always had regular menstruation at twenty-eight day intervals with moderate flow and without pain. The uterus contained a 7 mm. subserous leiomyoma and was moderately enlarged. The myometrium was found to contain 22 per cent fibrous tissue in the submucous layer. The second exception was a 42 year old multipara sexigravida whose uterus measured 6 by 5 by 3.5 cm. A cervical repair done nine months previously, after the birth of her last child, had been followed by cervical stenosis with severe dysmenorrhea but without menorrhagia. Her uterus contained 25 per cent fibrous tissue in the submucous layer.

The character of the menorrhagia in the remaining 25 cases was described as "heavy," "profuse" or "flooding" and lasted for periods varying from that of the patient's customary duration to twenty days. In 1 case hysterectomy was performed ten days after the onset of severe menorrhagia, but the majority of the patients had had the symptoms for from two to three years, while the longest duration of symptoms was seven years. The periods remained regular and at former intervals in one third of the cases, while in the remainder they became irregular and occurred at shorter intervals.

In only 1 patient did dysmenorrhea develop with the menorrhagia, while in 2 others the menstrual pain usually experienced was increased in severity and became cramplike in character.

None of the patients had metrorrhagia.

In some cases the date of the last delivery antedated the onset of symptoms by twenty years. In only 1 instance was the last delivery within the same year as the onset of symptoms.

Prior Therapy.—In a few of the cases estrogens had been administered without change in symptoms.

Preoperative Clinical Diagnosis.—The clinical diagnosis in these 27 cases were: leiomyoma in 21, adenomyosis in 1, stenosis of the cervix in 1. A preoperative diagnosis of fibrosis uteri was made correctly in the remaining 4 cases.

On pathologic examination one or more additional lesions were found in 15 of the 27 cases. In 11 cases these lesions were subserous or intramural leiomyomas (measuring as much as 7 mm.); in 2, adenomyosis; in 3, small endometrial polyps, and in 2, cervical polyps. With the possible exception of the endometrial polyps, these additional lesions were not considered adequate to explain the patient's severe menorrhagia or the consistency of the myometrium.

Gross Appearance of Uterus.—In 1 case the uterus was normal in size, measuring 5 by 4 by 3 cm. In all the remaining cases it was enlarged; the average measurements, excluding cervix, being 7.2 by 6.1 by 3.8 cm.; the largest measured 11 by 7.5 by 6 cm. The outline was normal. The myometrium averaged 2.6 cm. in width (extremes, 1.5 to 4 cm.; normal, 1 to 1.3 cm.). The color was pale pink to dull white. The fresh cut surface was either flat or bulged slightly, and, except for the prominent vessels in the vascular layer, was finely textured in contrast to the coarsely trabeculated bulging cut surface of a uterus with adenomyosis. The consistency was increased in all cases but not always uniformly; that is to say, it was increased to a moderate to marked degree in the inner one third to one half of the myometrium in every case and was increased in the outer half in only half the series, being normal beyond the vascular layer in the remainder. The endometrium showed no notable changes.

On the basis of the menstrual history, the hysterectomy was performed within a week of the next menstrual period in 2 cases, in the first week following menstruation in 1, in the second week in 5 and in the third week in 6, while in the remainder it was performed during a period of menorrhagia continuous with or part of the menstrual period.

Microscopic Appearance.—In the normal myometrium the smooth muscle fibers are collected into various-sized bundles which interlace in all directions. Separating the muscle bundles are variable amounts of fibrous tissue, while surrounding each individual muscle fiber is a thin connective tissue sheath. This connective tissue is made up of collagen fibers, elastic fibers and reticulum fibrils. In fibrosis uteri an abnormal amount of connective tissue occurs both between the muscle bundles and, more characteristically, around the individual muscle cells. As well as could be determined, this increase is in collagen and not in elastic fibers or reticulum. Most of the cases fell into Shaw's class 2. This increase of connective tissue is, in some cases, apparent on staining with hematoxylin and eosin, but more often Mallory's or Masson's stain is necessary to demonstrate the abnormality, while occasionally accurate measurement is essential for a diagnosis.

The results of the measuring of the fibrous tissue content of the myometrium are shown in the accompanying table. There is an appreciable increase in the fibrous connective tissue throughout the myometrium in fibrosis uteri as compared with controls; however, there is considerable overlapping of this content in the two series with respect to the figures for myometrium as a whole as well as those for the vascular and supravascular layers. A significant and diagnostic difference, however, occurs in the submucous layer: In all of the cases of fibrosis this layer contained 15 per cent or more fibrous tissue, while in all but 6 of the

controls it contained less than 15 per cent. In 3 of the exceptions it contained 15 per cent, and in 3, 17, 21 and 23 per cent, respectively. Of the 6 women represented in these exceptions, 2 were multiparous, 3 primiparous and 3 nulliparous. One of these patients, aged 52, was nearing the end of the menopause. Her uterus was removed for multiple large tumors, diagnosed as leiomyoma. She did not complain of menorrhagia. Another had hysterectomy for acute endometritis, cause undetermined, in the first trimester of pregnancy. This patient had had mild menorrhagia, preceding the pregnancy, and her condition might conceivably represent an early stage in the development of fibrosis uteri. Three additional women had hysterectomy for leiomyoma and the last for extensive cervical laceration. No factor common to these 6 patients could be found; their ages varied from 21 to 52 years.

That the major localization of the fibrosis is in the submucosal layer is particularly interesting in view of the studies of Faulkner[11] who, while investigating the blood supply of the uterus, found the submucosal

Fibrous Tissue Content of Myometrium

	Submucous Layer *	Vascular Layer *	Supravascular Layer *	Entire Myometrium *
Fibrosis uteri				
Average	21.9	24.9	33.6	27.7
Extremes	15-35	11-33	8-50	19-37
Controls				
Average	11.6	16.7	28.6	20.0
Extremes	3-23	9-27	13-42	11-27

* The value is the percentage of the width.

zone poorly injected during the follicular phase of the cycle but well injected during the luteal phase, as well as in those patients bleeding at the time of surgical treatment. This probably explains the difference in appearance between Shaw's classes 1 and 3, and 2 and 4, since we noted in our own cases that the fibrous connective tissue, whether it is interfascicular or intrafascicular, appeared looser during the luteal phase of the cycle than during the follicular phase. However, in our cases the consistency of the myometrium and the actual amount of fibrous tissue present in it were definitely increased, regardless of the phase of the menstrual cycle during which the uterus was removed.

William and Kinney concluded that the increased consistency of the myometrium in fibrosis uteri is due to hypertrophy of the individual muscle fibers as measured in sections stained with hematoxylin and eosin. We found that the distinction between the muscle cell and its fibrous sheath with this stain was poor, so that measurement of cell diameters

11. Faulkner, R. L.: Am. J. Obst. & Gynec. **49**:1, 1945.

was carried out on sections stained with Mallory's or Masson's stain. In only 3 of the 27 cases of fibrosis uteri was the cell diameter found increased as compared with the controls. In these 3 cases the fibrous tissue content of the submucous layer was 15, 18 and 26 per cent, respectively.

In addition, the muscle fibers present within a space 158 microns wide were counted, and the only cases of fibrosis uteri in which the fibers in this space were significantly fewer as compared with the controls were the 3 instances aforementioned in which muscle fibers were hypertrophied.

In no case were leukocytes found outside the vessels in the myometrium.

Adnexal Lesions.—The tubes and ovaries, whether examined in situ at operation or grossly after being removed with the uterus, revealed no lesion present in a sufficient number of cases to be considered of importance in the etiology of the uterine changes.

SUMMARY

The patient with fibrosis uteri may be described as having a history of profuse menorrhagia without metrorrhagia and usually without dysmenorrhea. Her age may be any age between 30 and 55 years. She may be nulliparous or primiparous but most commonly is multiparous. On pelvic examination the uterus is found symmetrically enlarged and in normal position. The excised uterus is revealed as a slightly to moderately enlarged organ. The myometrium is pale, finely textured, and thickened, with consistency definitely increased throughout or at least in its inner half. On microscopic examination an increase of fibrous tissue is observed in the submucous layer of the myometrium equaling or exceeding 15 per cent of the width of this layer and appearing as an increase of both the interfascicular and the intrafascicular connective tissue.

This study does not reveal the exact causation or pathogenesis of the lesion but does provide ample evidence that it is not an end stage of chronic metritis, that hypertrophy of muscle fibers plays a minor role (occurring in only 11 per cent of the cases) and that it is not a form of subinvolution of the uterus.

CONCLUSION

From the data presented, fibrosis uteri may be considered a fairly well delimited clinical and pathologic entity. The lesion consists of fibrosis of the myometrium, most marked in the submucosal area, a lesion which at the same time separates this entity from simple muscular hypertrophy and subinvolution and establishes a defect of the myometrium as a causative factor in menorrhagia.

PARATHYROID ADENOMA WITH GENERALIZED METASTATIC CALCIFICATION

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RECORDED instances of metastatic calcification associated with an adenoma of one of the parathyroid glands are few. According to Mulligan,[1] there are only 16 recorded cases of metastatic calcification in which necropsy observations are available. Hyperparathyroidism usually produces characteristic, though varied, clinical manifestations, and therefore may be recognized clinically. In this paper, a case is reported in which signs and symptoms suggestive of hyperparathyroidism were present, yet the condition was not identified until the postmortem examination was completed. Because of the diagnostic problem encountered and the rarity of generalized metastatic calcification, this case is presented in some detail.

REPORT OF A CASE

G. F., a white man aged 49, was apparently well until April 1945, when he began to have frequent episodes of vomiting, not related to meals. A few days later, frequency of urination was noted and nocturia, with micturition up to eight times a night. The urine seemed "unusually warm." A dull lumbar ache and vague pains of the arms and legs were noted. The patient continued working until May 13, when he became mentally confused. His physician treated him with penicillin for "bad teeth and pus in the urine."

The symptoms persisted, the mental confusion increased, and on May 23 the patient was sent to a psychiatric institution. After intravenous administration of dextrose in saline solution and Hartmann's solution, his mental state seemed to improve. On May 29 he was transferred to the University of Oklahoma Hospitals. During this admission the highest blood pressure recorded was 146 systolic and 80 diastolic. On one occasion the blood nonprotein nitrogen was 67 mg. per hundred cubic centimeters. Occasional granular casts were noted in the urine, but no albumin was detected. The specific gravity ranged from 1.008 to 1.022. Up to 30 white blood cells and 40 red blood cells per high power field were counted in the urine at various times. He was discharged June 30, with the diagnosis of subsiding acute glomerulonephritis, improved but unable to resume his work as

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1. Mulligan, R. M.: Arch. Path. **43**:177, 1947.

a carpenter. One month before his readmission, Nov. 6, 1946, a cold and cough developed, soon followed by increased weakness and pain of the arms and legs.

At the time of readmission he was well developed, well nourished and appeared subacutely ill. The sensorium was somewhat clouded. His temperature was 99 F. The retinal vessels were tortuous; there were no hemorrhages. The teeth were carious, and there was marked alveolar pyorrhea. Just above the manubrium there was a firm mass, 2 cm. in diameter, apparently attached to the trachea. The lung fields were clear to auscultation and percussion. A soft systolic murmur was heard over the tricuspid, aortic and pulmonic valve areas. The pulse rate was 76, with the rhythm regular. The blood pressure was 168 systolic and 90 diastolic. The remainder of the physical examination yielded no pertinent information.

The urine was acid, with a specific gravity of 1.008, a trace of albumin and no glucose; microscopic examination revealed a few red blood cells and epithelial cells per high power field and no white blood cells or casts. The red blood cell count was 5,260,000; the hemoglobin content was 14.5 Gm.; the white blood cell count was 12,750, with neutrophils 71, lymphocytes 26 and monocytes 3 per cent. The plasma proteins were 6.9 Gm. per hundred cubic centimeters, with an albumin-globulin ratio of 5.1 : 1.8. The blood nonprotein nitrogen was 102 mg. per hundred cubic centimeters. Examination of the spinal fluid gave negative results. Roentgenograms of the chest revealed no significant changes.

Symptomatic medications and intravenous injections of fluids were administered. Frequent injections of a morphine salt were required for relief of the chief complaint, pain of the arms and legs. The blood nonprotein nitrogen fluctuated between 35 and 150 mg. per hundred cubic centimeters. The patient's condition became progressively worse, and on November 18 the restlessness and disorientation were marked. Rales were heard throughout both lung fields. The pulse was feeble; the rate, 130. The blood pressure was 100 systolic and 70 diastolic. The red blood cell count was 3,660,000; the hemoglobin content, 11 Gm. The plasma proteins were 5.8 Gm. per hundred cubic centimeters, with an albumin-globulin ratio of 3 : 2.8. The patient vomited frequently for several hours on the day of his death, November 20. The clinical impression was chronic glomerulonephritis with terminal uremia.

Necropsy (seven hours after death).—The body was 167 cm. long and weighed approximately 150 pounds (68 Kg.). A thin brown liquid oozed from the mouth. The abdomen was slightly distended. No other changes were noted on external examination.

The peritoneal cavity contained no excess fluid. The stomach was distended with air and 500 cc. of a thin brown liquid. The entire duodenum and the proximal 16 cm. of jejunum were distended up to 5 cm. in diameter and contained a thin brown liquid. Distal to this portion of the jejunum, a segment 12 cm. long was thin, friable, discolored red-gray-black and covered with fibrin. The branch of the superior mesenteric artery supplying this segment was firm and filled with a thrombus. Distal to the infarcted portion the distention lessened gradually and disappeared in the ileum.

The pleural and pericardial cavities contained no excess fluid; their surfaces were smooth and glistening. The heart measured 8.5 cm. from base to apex and 9 cm. across the base; it weighed 375 Gm. There were no valvular lesions. The trachea and bronchi were filled with a thin brown liquid similar to that in the stomach and the oral cavity. The posterior portions of the lungs were lumpy; the cut surfaces were mottled red and gray. The left lung weighed 775 Gm. and the right 1,000 Gm.

The spleen weighed 170 Gm. and the liver 1,900 Gm.; they were otherwise unremarkable. Cholesterosis with some polyp-like projections was noted in the gallbladder.

The left kidney measured 13 by 7 by 5 cm. and weighed 225 Gm. The cut surfaces did not bulge and were pale. The cortical and medullary markings were indistinct. The capsule stripped with slight difficulty, leaving a roughened, gray-pink surface. The mucosa of the calices, pelvis and ureter was pale and delicate. There were no calculi. The right kidney resembled the left and weighed 210 Gm. The urinary bladder contained some cloudy yellow urine and no concretions. Its wall was of usual thickness, and the mucosa was slightly injected.

In the isthmus of the thyroid gland there was a partly calcified nodule, 2 cm. in diameter; two softer nodules were contained in the lower pole of the right lobe. On the anterior surface of the body of the first thoracic vertebra, immediately to the left of the esophagus, there was an encapsulated pink-yellow soft mass. It was not connected with the thyroid gland, the thymus, or other nearby structures. The mass was fusiform, 6 by 2.5 by 1.5 cm., and weighed 28 Gm. The cut surfaces were mottled pink and yellow; they showed cavities measuring as much as 0.4 cm. in diameter, containing clear fluid (fig. 1).

The pancreas, the adrenal glands and the genital organs were not remarkable. No gross abnormalities of the skeleton were apparent. Examination of the cranial contents was not authorized.

Microscopic preparations stained with hematoxylin and eosin revealed that the mass from the left posterior part of the superior mediastinum was a parathyroid adenoma (fig. 2). Broad sheets of cells having large compact or vesicular round nuclei with a halo of cytoplasm or with indistinct cytoplasmic borders (chief cells) were seen in a scanty connective tissue stroma containing the blood vessels. There were scattered occasional cells with a pink-stained cytoplasm (oxyphil cells). No acinous structures were seen. Within the substance of the mass there were some empty spaces bordered by compressed cells or a delicate membrane-like line. In the scanty connective tissue stroma there were streaks of deposits stained lavender or blue. The lumens of occasional blood vessels were obliterated and in part recanalized (fig. 3).

In the heart, focal areas of lavender-stained deposits were seen in muscle fibers, in the connective tissue and in the walls of blood vessels (fig. 4). In the lungs, similar deposits were seen in some of the septums and in blood vessels (fig. 5). The calcific changes were marked in the capsule, the septums and the blood vessels of the spleen.

In the kidneys the renal pattern was partly obliterated, with some glomeruli hyalinized or replaced by lavender-stained granular deposits. Some glomeruli had thickened Bowman's capsules (fig. 6). The tubules surrounding the hyalinized glomeruli were few, and the stroma contained lavender-stained deposits and an infiltrate of lymphocytes, plasma cells and large mononuclear cells. Elsewhere, occasional glomeruli appeared intact, and the tubules surrounding them were distended. Only occasional blood vessels were intact. Many contained calcific deposits in their walls and thrombi in their lumens.

The stroma and the blood vessels of the stomach, the adrenal glands and the lymph nodes also contained focal calcific deposits.

In the three adenomas of the thyroid gland, particularly in the one located in the isthmus, deposits stained lavender or blue seemed to replace acini or groups of acini. They were also seen in the ground substance of the septums and within the walls of blood vessels.

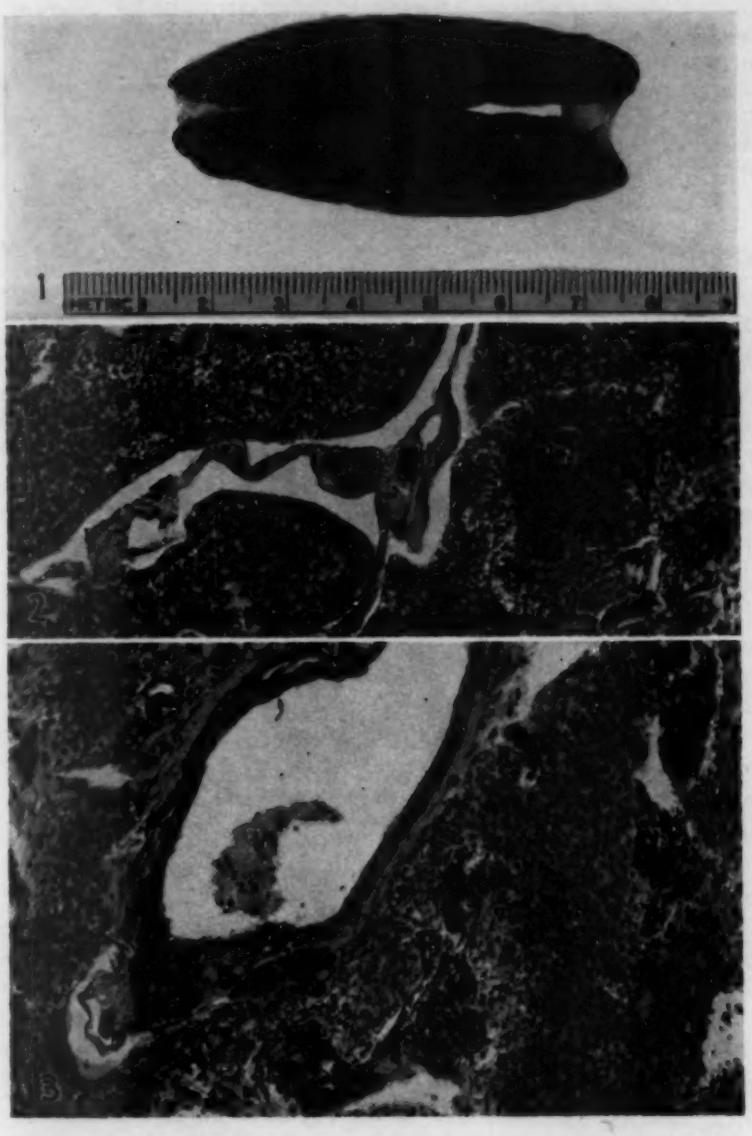

Fig. 1.—Cut surfaces of the adenoma of the parathyroid gland located in the left posterior part of the superior mediastinum. It measured 6 by 2.5 by 1.5 cm. and weighed 28 Gm.

Fig. 2.—Microscopic appearance of the parathyroid adenoma; × 125. Broad sheets of cells having large compact or vesicular nuclei in a halo of cytoplasm are seen in a scanty connective tissue stroma.

Fig. 3.—Parathyroid adenoma; × 125. In the scanty connective tissue stroma there are streaks of calcific deposits stained lavender or blue. The lumens of occasional blood vessels are obliterated and in part recanalized.

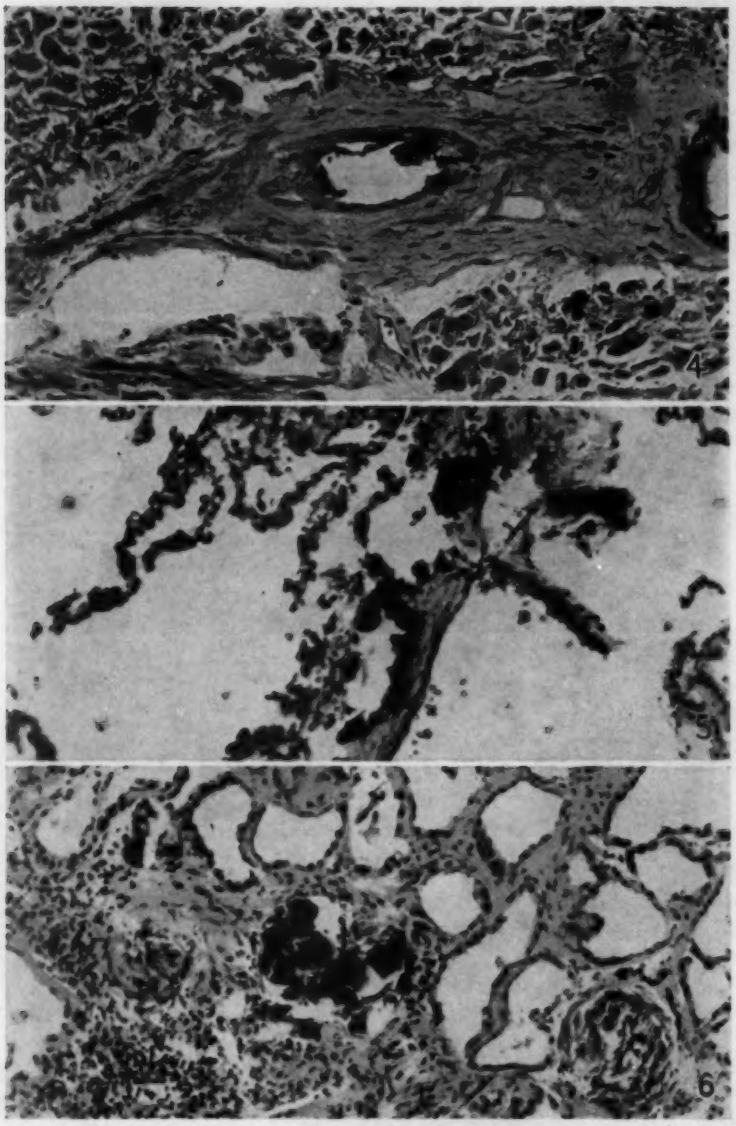

Fig. 4.—Metastatic calcification of the myocardium; × 125. Areas of lavender-stained calcific deposits are seen in muscle fibers, in the connective tissue and in the walls of blood vessels.

Fig. 5.—Metastatic calcification of the lungs; × 125. In the septums and the blood vessels of the lung there are calcific deposits similar to those in the myocardium.

Fig. 6.—Metastatic calcification of the kidneys; × 125. The renal pattern is partly obliterated, with some glomeruli hyalinized or replaced by lavender-stained granular deposits.

The calcific deposits were present in the aorta and in arteries within all the viscera examined with the exception of the liver. Lavender-stained deposits were seen in the intima and the inner portion of the media. The lumen of the artery supplying the infarcted segment of the jejunum contained a thrombus. Irregular streaks of lavender-stained granular deposits were seen inside and outside the frequently interrupted inner elastic lamina of the media. Neutrophilic granulocytes had infiltrated the media, the adventitia and the surrounding adipose tissue, which was spread apart.

COMMENT

According to Norris,[2] 322 cases of adenoma of the parathyroid glands were reported between 1903 and 1945. One of the inferior pair of glands was the site of the adenoma in 83.8 per cent of the cases. The adenoma was in an aberrant location in 10.7 per cent of the cases, and the location was in the mediastinum in 63.3 per cent of this group. Thus, a parathyroid adenoma located in the mediastinum, as in the case herein reported, is uncommon, observed in less than 7 per cent of the recorded cases. Adenoma of the parathyroid glands is rarely palpable. When located in the mediastinum it is even less accessible. The observation that the inferior pair of parathyroid glands may be aberrant in the neck and the mediastinum is explained by the fact that they are in close relationship with the primordia of the thymus at the time at which they are derived from the third pharyngeal pouch and are descending in the neck during embryonic life. Because of this close relationship, the inferior pair of parathyroid glands was termed by Weller[3] the "parathymus glands." In addition to the developmental factor responsible for the aberrant locations, Cope[4] suggested a contributing factor. He believed that an enlarging adenoma meets less resistance in growing downward and thus may come to lie in either the anterior or the posterior part of the superior mediastinum. Such a growth usually remains connected to its site of origin by an elongated vascular pedicle.

According to Mulligan,[1] the four underlying causes of metastatic calcification are, in order of frequency, skeletal disease, chronic renal disease, neoplasms of the parathyroid glands and hypervitaminosis D. The organs most frequently involved in the calcific process are the kidneys, the lungs, the heart, the systemic arteries and less frequently the stomach and other organs. Mulligan reviewed the factors involved in the deposition of the calcium found in the soft tissues. When this is due to the activity of a parathyroid adenoma, the chief factor responsible is apparently the state of the blood, which is supersaturated with calcium withdrawn from the skeleton. Sites favorable to such deposition are

2. Norris, E. H.: Internat. Abstr. Surg. **84**:1, 1947.

3. Weller, G. L. Jr.: Contrib. Embryol. **24**:95, 1933; Publication 443, Carnegie Institution of Washington, 1933.

4. Cope, O.: Ann. Surg. **114**:706, 1941.

those with a low hydrogen ion concentration. Organs supplied with blood of low carbon dioxide or high oxygen content (lungs, heart, systemic arteries) and organs which excrete acids (kidneys, stomach, lungs) have a relatively low hydrogen ion concentration. The tissue injury due to the initial calcific change and the degree of phosphatase activity are additional factors to be considered.

The average duration of the symptoms of hyperparathyroidism due to an adenoma is probably five to seven years, according to Norris,[2] who stated that few cases had been diagnosed with symptoms present less than two years. In our case the patient was well until nineteen months before his death.

In retrospect, the patient had symptoms typical of hyperparathyroidism, such as weakness, pains of the back and extremities, and also urinary complaints. The latter, however, were not the kind usually produced by renal calculi. The mental confusion may have been caused by the hypercalcemia or by the multiple thrombosis of small cerebral vessels.

The hypercalcemia, undoubtedly present, though its degree was not determined, must have had its source in the skeleton. Review of the only roentgenograms taken, those of the chest, disclosed no osteoporosis. At necropsy no obvious changes were noted in the skeleton.

Microscopic deposits of calcium were observed in the kidneys, heart, lungs, stomach, spleen, adrenal glands, lymph nodes and their blood vessels. They were also observed in the adenomas of the thyroid gland and within the stroma of the parathyroid adenoma. The calcium deposited in glomeruli, tubules, stroma and blood vessels must have seriously interfered with renal function. The calcium deposited in a branch of the superior mesenteric artery caused formation of a thrombus. This in turn caused infarction of the jejunum with early peritonitis, distention of the proximal jejunum, the duodenum and the stomach, and vomiting. Aspiration of the vomitus aggravated the pneumonia, which was the immediate cause of death.

SUMMARY

Extensive metastatic calcification occurring in a white man aged 49 with hyperparathyroidism is reported. It was caused by an adenoma of the left inferior parathyroid gland, which was located in the posterior part of the superior mediastinum. The condition was not diagnosed during life. Death was due to the sequelae of thrombosis of a branch of the superior mesenteric artery caused by calcific change in its wall.

RETICULOENDOTHELIAL CELLS REACTING TO TOXIC ANTIGENS AND TO INFECTION

P. BUENO, V.M.D.
SÃO PAULO, BRAZIL

IN A PREVIOUS PAPER [1] I have shown that normal horse serum intraperitoneally injected into guinea pigs sensitized to this antigen causes lesions in certain elements of the reticuloendothelial system. This phenomenon was observed in the Kupffer cells of the liver and in the germinal cells of the lymph node cortex and of the lymph follicles of the spleen.

In further studies changes were found in these same elements after the injection of bacterial extracts. This observation led me to make systematic investigations in this direction. Thus the results reported here refer to studies of lesions observed in reticuloendothelial cells in various organs after injection of toxic antigens or during an infectious process.

MATERIAL

Guinea pigs weighing between 250 and 450 Gm. were used, and the following antigens were injected: extract of Salmonella paratyphi A (a filtrate of disintegrated bacteria in isotonic solution of sodium chloride), brucellergin (prepared according to the Huddleson technic), tetanus toxin (200,000 minimal lethal doses [mice] per cubic centimeter), diphtheria toxin (1/1500 minimal lethal dose) and living Bacillus anthracis. Most of the injections were subcutaneous, but the first two antigens were also introduced by the intracardiac route.

The 49 guinea pigs used in this experiment were divided into groups as follows: 11 guinea pigs were given brucellergin in doses of 1 and 2 cc.; 8 received salmonella extract in doses of 1 to 5 cc.; 10 were given tetanus toxin in doses of 1 cc. of a 0.4: 1,000 dilution and 0.5 and 1 cc. of undiluted toxin; 8 received 0.1 and 0.2 cc. of diphtheria toxin, and 12 animals were infected with 0.5 cc. of B. anthracis suspension in isotonic solution of sodium chloride.

The animals which received the first four antigens were either killed or died, most of them within seven, twenty-four and thirty hours after the injection. The deaths of those infected with B. anthracis occurred from two to four days after the inoculation.

A large series of normal guinea pigs and one of guinea pigs given injections of nontoxic antigens were studied as controls. For the latter such antigens as egg albumin and normal horse serum were used.

From the Department of Pathology, Instituto Biológico.

1. Bueno, P.: Arch. Path. 42:412, 1946.

From the animals given injections the following material was taken and studied histologically: the lymph nodes, the spleen, the liver and the bone marrow. The material was fixed in solution of formaldehyde U.S.P. diluted 1:5, embedded in paraffin and sectioned and the sections were stained with hematoxylin-eosin.

HISTOLOGIC OBSERVATIONS

Liver.—In most cases histologic study revealed that heavy damage had occurred in a great number of the Kupffer cells.

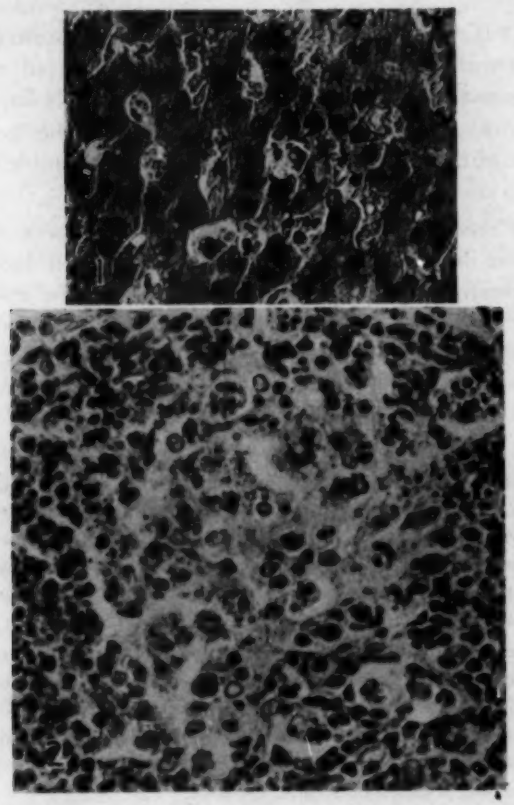

Fig. 1.—Liver after a subcutaneous injection of diphtheria toxin. Note the disintegrating Kupffer cells. × 350.

Fig. 2.—Lymph follicle of spleen showing regressive alterations. Note nuclear pyknosis and cell particles. × 420.

It was observed that the initial cell change is a marked nuclear tumefaction; at first the nucleus is enlarged and slightly stained; stain affinity is increased later, and the beginning of necrobiosis is observed. Then successive phases of contraction, hyperchromatosis and finally nuclear disintegration can be noted (fig. 1). More rarely disintegration of nuclei is seen during the state of tumefaction. In some cases these cells present evident decrease in number, and only cell particles can be seen in their place.

Spleen.—The spleen was also observed to be a site of lesions. These occurred, however, only in the lymph follicles, where evident necrobiotic changes were found (fig. 2). Cells with enlarged or pyknotic nuclei, as well as cell particles, either dispersed or within phagocytes, were noted. It seemed that only germinal cells or slightly differentiated cells presented lesions. The more developed elements did not show changes.

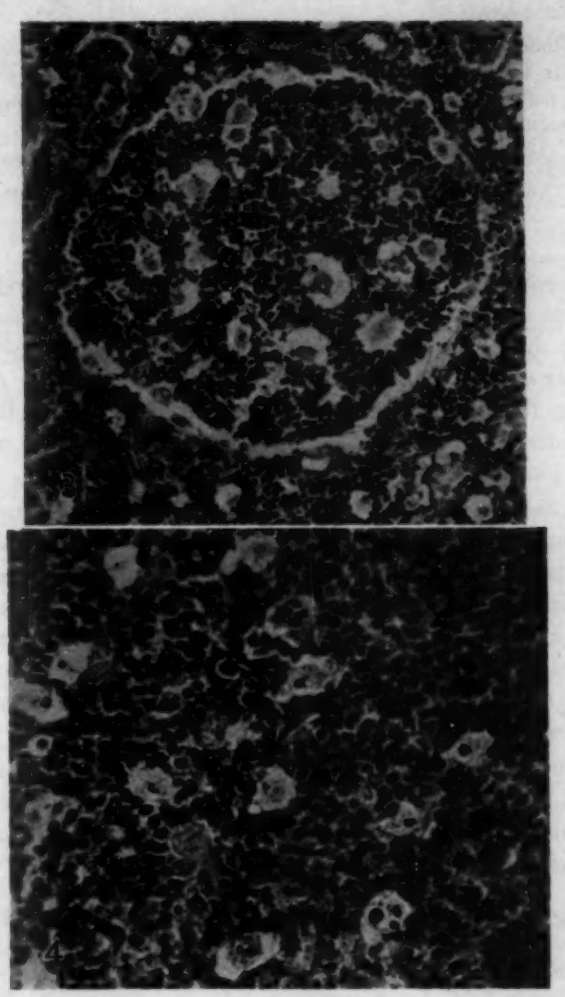

Fig. 3.—Lymph follicle of a lymph node after B. anthracis infection. It shows germinal cells in a state of necrobiosis. × 200.

Fig. 4.—Lymph node after a subcutaneous injection of diphtheria toxin. Extrafollicular germinal cells are seen in a state of necrobiosis. × 400.

No lesions were observed in the reticuloendothelial cells of the pulp. In these cells only nuclear tumefaction was noted.

Lymph Nodes.—The lymph nodes had cell lesions, but these seemed to be confined to the follicular and extrafollicular germinal cells of the cortex.

Generally, marked structural changes appeared within the germinal centers, where the undifferentiated cells or the elements that were in an early stage of differentiation showed distinct necrobiotic changes (fig. 3). Cells with nuclear tumefaction or pyknosis could be seen besides other elements that were already disintegrated. Marked cellular rarefaction was therefore evident, which in more advanced phases implicated the whole follicle. Sometimes almost all the cells completely disappeared.

The extrafollicular germinal cells which are scattered throughout the cortex showed similar lesions. The nuclei of some were enlarged, and the cytoplasm contained cell particles or the cells presented necrobiosis, in sharp contrast with the surrounding normal tissue (fig. 4).

No significant changes were found in other reticuloendothelial elements.

Bone Marrow.—No lesions were observed in cells of this tissue.

COMMENT AND CONCLUSIONS

The present observations show that certain cells of the reticuloendothelial system are damaged when toxic antigens are introduced into the organism or during infection. Particularly interesting, however, is the fact that was emphasized initially, that these are the same cells which react during the anaphylactic phenomenon. This shows that certain undifferentiated mesenchymal cells have a special capacity of reacting to different kinds of antigenic stimuli.

On the other hand, the results confirm a series of researches which indicated that toxins damage lymphatic tissue. My observations showed, moreover, that lesions occur not only in the germinal elements of the lymphatic tissue but also in cells which are considered as a source of monocytes or histiocytes (for instance, extrafollicular germinal cells of the cortex of the lymph nodes and the Kupffer cells). Therefore, it is evident that there is a similar way of reaction among the precursors of mononuclears.

Finally, it is interesting to consider that these findings, which demonstrate a kind of electivity of antigens for certain undifferentiated or germinal reticuloendothelial cells, seem to accord with the concept that these cells function as producers of antibodies.[2]

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2. Bunting, C. H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 1, p. 439. Epstein, E.: Virchows Arch. f. path. Anat. **273**:89, 1929. Bueno.[1]

NUCLEOLAR SUBSTANCE IN THE ANTERIOR LOBE OF THE HUMAN PITUITARY GLAND

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A NUMBER of investigators [1] have suggested that both in man and in a number of animals the nuclei of the glandular epithelium of the pituitary gland take part in the process of cellular secretion. Erdheim and Stumme [2] called attention to the presence of huge vesicles of nucleolar origin in the pituitary gland of the pregnant woman. Gellerstedt and Lundquist [3] and shortly afterward Mellgren [4] pointed out peculiar cells in the pituitary body in cases of basophilism and of adrenogenital syndromes. These cells were characterized by an intranuclear acidophilic vesicular formation which at times reached such a size as to almost completely cover the nuclear area. Cells corresponding to the descriptions of these investigators were described by Romeis [1f] as observed in human pituitary glands otherwise considered to be normal.

The results of a further investigation of the intimate cytologic structure of these intranuclear formations, their frequency, their relation to age and to various pathologic conditions and significance are here briefly presented.

MATERIAL AND METHODS

The material submitted to examination consisted of 404 consecutive pituitary glands obtained at postmortem examinations; 200 belonged to males and 204 to females. Persons of all ages were represented in the group, including 10 fetuses from the fourth to the eighth month. Except for 5 normal persons, whose deaths were due to violence, all the persons from whom pituitary glands were taken died of pathologic conditjons, some of which involved one or more of the endocrine

From the Anatomopathological Institute of the University of Milan (Dr. P. Redaelli, director).

1. (*a*) Pirone, R.: Arch. di fisiol. **2**:60, 1905. (*b*) Guerrini, G.: Sperimentale, Arch. di biol. **58**:837, 1904. (*c*) Trautmann, A.: Frankfurt. Ztschr. f. Path. **18**: 173, 1915. (*d*) Bock, F.: Ztschr. f. Zool. **131**:645, 1928. (*e*) Kirkman, H.: Am. J. Anat. **61**: 233, 1937. (*f*) Romeis, B.: Hypophyse, in von Mollendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1940, vol. 6, pt. 3, p. 597.

2. Erdheim, J., and Stumme, E.: Beitr. z. path. Anat. u. z. allg. Path. **46**:1, 1909.

3. Gellerstedt, N., and Lundquist, R.: Upsala läkaref. förh. **45**:233, 1939.

4. Mellgren, J.: Beitr. z. path. Anat. u. z. allg. Path. **106**:482, 1942.

glands. The material studied is therefore being divided into "endocrine" and "nonendocrine" groups.

The pituitary bodies were removed from five to eighteen hours after death, immediately fixed in 5 per cent formaldehyde solution or in Zenker or Susa solution, embedded in paraffin and cut in sections 5 microns thick, according to a horizontal plane. The sections were stained with hematoxylin-eosin, iron-hematoxylin (Mallory) and cresofuchsin-azocarmine. In a number of cases the study was conducted on serial sections practically including the entire gland. A magnification of 480 to 600 diameters has been found most suitable for the detection of the formations under consideration.

OBSERVATIONS

Cytologic Study.—Vesicular nucleolar-like structures were seen inside the nuclei in 34 per cent of the pituitary glands examined. The cells displaying this peculiar pattern were consistently found in the anterior lobe; they ranged in number from 1 to 5, less frequently from 5 to 10 and in exceptional cases from 50 to 120 per section.

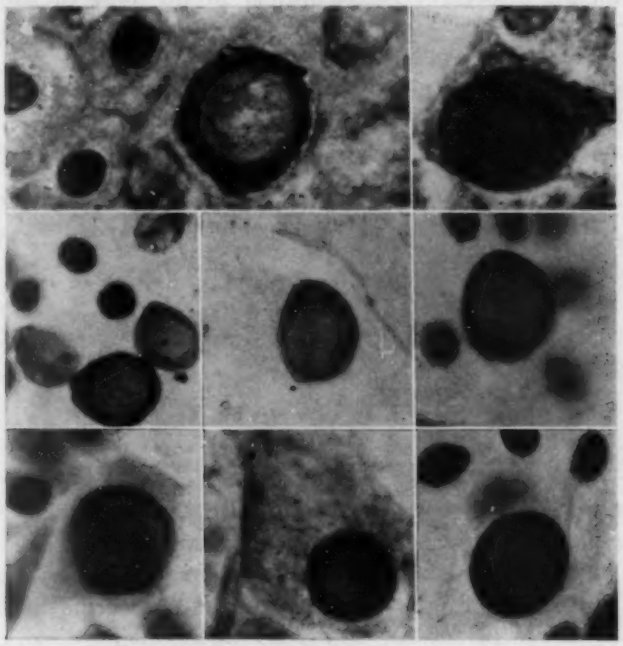
In the sections stained with hematoxylin and eosin these cells (which from now on, for the sake of brevity, will be called X cells) stood out conspicuously because of their notable size and the characteristic globular appearance of the large, intensely acidophilic formation within the nucleus; at other times, however, they could be detected only after careful investigation, as they did not appear larger than the surrounding epithelial cells (figure). In no instance were X cells recognized in the posterior lobe or in the pars intermedia, and most of them appeared to be in the marginal and posterior portions of the anterior lobe, often where this lobe joined the intermediate lobe.

As far as size and shape are concerned, the cells ranged from 10 to 35 microns in diameter and from round to ovoid. The cytoplasm was abundant, spongy in appearance, palely stained and usually amphophilic, with variations from slightly acidophilic to slightly basophilic. In the vacuolated cytoplasm a few thin granules, of rather uniform size, were recognizable. The granules stained pink with eosin, pale yellow with orange G and from gray to violet with the cresofuchsin. The majority of the X cells had characteristics of chromophobe cells; in some cases, however, identical endonuclear inclusions were seen in basophilic degranulated cells and occasionally in typical eosinophilic cells. In pregnancy —also in other conditions showing pregnancy-like changes—endonuclear inclusions were found in pregnancy cells.

The nucleus occupied a good portion of the cell, measuring from 15 to 22 microns in diameter. It varied in shape from round to ovoid, with marked irregularity of the contours. When the nuclear inclusion was small, the chromatin of the nucleus was disposed in a delicate net, thickened at the nodal points and at the inner aspect of the nuclear membrane; however, when the inclusion was so large as to cover most of the nuclear area, the substance proper of the nucleus was reduced

to a narrow rim, and the chromatin appeared in the form of large blocks, well separated one from the other. Cells with two nuclei were encountered occasionally, only one of the two nuclei displaying evidence of nuclear inclusion.

The intranuclear formation itself ranged from 10 to 20 microns in diameter and appeared like a vesicle, clearly outlined, compact and more or less intensely acidophilic, usually homogeneous but sometimes with flakelike or granule-like colorless lacunar spaces. It stained pink with eosin, orange-yellow with orange G and bright red with azocarmine. Thionine and toluidine blue did not stain it metachromatically; it was not stained by mucicarmine; it gave neither the iron



Cells of the anterior lobe of the human pituitary gland with giant nucleoli ("X cells"). Hematoxylin-eosin; × 1,000.

reactions nor those of fats or lipids. Bauer's reaction for the glycoproteins appeared to be only slightly positive.

Relation to Sex and Age.—The X cells are not constantly found in the anterior lobe of the human pituitary body, their appearance showing close relations with age and with certain physiopathologic conditions. The incidence of the finding in the two sexes and in the different age groups is shown in table 1.

In only 34 per cent of the 404 pituitary bodies examined could X cells be detected. No differences were noticed in them between the

two sexes; the slightly higher percentage of females whose pituitary glands contained these cells lacked statistical meaning.

In none of the 51 pituitary bodies of prepubescent persons, including fetal and neonatal specimens, was it possible to detect a single X cell. The highest percentages have been observed at the time of sexual maturity in both sexes. In the climacteric and the postclimacteric period the finding decreased in frequency both in males and in females, to rise again in males beyond the seventh decade of life.

Relation to Physiopathologic Conditions.—The material has been divided into two groups, one including pituitary bodies of persons with endocrine disorders and the other those of persons who did not have endocrine disorders; in the former group those of women who died during pregnancy and puerperium have been included.

TABLE 1.—*Relation of Cells Containing Endonuclear Inclusions to Sex and Age.*

Subjects	Age	Percentage of Males Whose Pituitary Glands Revealed X Cells	Percentage of Females Whose Pituitary Glands Revealed X Cells	Percentage of Total Group Whose Pituitary Glands Revealed X Cells
10	Under 1 yr.	0	0	0
41	1-10 yr.	0	0	0
21	11-20 yr.	35	43	38
25	21-30 yr.	60	65	64
53	31-40 yr.	35	56	47
73	41-50 yr.	37	39	38
80	51-60 yr.	24	26	25
56	61-70 yr.	21	22	21
35	Above 70 yr.	42	4	10
Total 404		33	35	34

A comparison between table 2, in which the "endocrine" group is analyzed, and table 3, in which the "nonendocrine" group is considered, shows a much higher incidence of X cells in the former than in the latter: 65 per cent (68 of 104 studied) in the "endocrine" group, compared with the 23 per cent (40 to 300) in the "nonendocrine" group.

The consistent finding of X cells in pregnancy and puerperium deserves special notice. The material represented primiparas and multiparas and stages from the third month of pregnancy to the fourth day of puerperium; death occurred because of puerperal septicemia, septic abortion, eclampsia, severe hemorrhage occurring after birth, placenta previa or high tracheal stenosis caused by a bulky retrosternal goiter.

The number of X cells seemed to increase with the advance of pregnancy. In the pituitary gland of a woman who died in puerperium four days after childbirth, as many as 100 X cells were found in a single section, and a good number of them appeared to be in the phase of emptying their secretion into the cellular cytoplasm. The intranuclear vesicle seemed to become progressively larger until it came

in direct contact with the nuclear membrane. At a further stage the vesicle was seen to empty its contents into the cytoplasm, this in absence of any evidence of an actual breaking through the nuclear membrane; after excretion of its contents the vesicle assumed a flakelike appearance, losing its individuality.

X cells were also consistently found in the pituitary glands of the 7 females listed as having undergone "female castration." This includes bilateral ovariectomy, total destruction of the ovaries due to bilateral cystic degeneration or to metastatic new growth (Krukenberg's tumor) and severe ovarian hypoplasia. All these patients were in the period of sexual maturity, their ages ranging from 32 to 50 years. X cells

TABLE 2.—*Relation of Cells Containing Endonuclear Inclusions to Endocrine Disorders*

Subjects	Pathologic Condition	Number with X Cells	Percentage with X Cells
19	Pregnancy and puerperium	19	100
7	Female castration	7	100
4	Hypogonadism of male (eunuchoidism)	4	100
1	Gynecomastia	1	100
1	Adrenogenital syndrome	1	100
7	Tuberculosis of adrenal gland with cortical hypofunction (Addison's disease)	7	100
5	Thyrotoxicosis	4	80
4	Hypogonadism of female	3	75
6	Prostatic hypertrophy	4	66
16	Gonadal neoplasms	10	62
8	Diabetes mellitus	2	25
18	Postclimacteric adiposity	5	27
4	Hyperostosis frontalis interna (Morgagni's syndrome)	1	25
4	Myasthenia gravis due to thymic tumor; hypopituitary cachexia (Simmond's disease); diabetes insipidus; pituitary dwarfism	0	0
Total 104		68	65

in great numbers were found in the anterior lobe of the pituitary gland of a 60 year old woman with the adrenogenital syndrome and an ovarian arrhenoblastoma.

The same appeared to be true among the males with gonadal insufficiencies, including eunuchoid adiposity and hypophysial gigantism and, in a single patient, gynecomastia associated with a femininizing neoplasm comparable to arrhenoblastoma. In the last case, that of a 57 year old man, the X cells were scattered in high number throughout the anterior lobe and showed patterns suggesting emptying of secretion.

In Addison's disease X cells were also constantly found but in a limited number; a concomitant numerical decrease and a pronounced degranulation of basophilic cells were noted, and an increase both in size and in number of chromophobe cells.

In 4 of 5 pituitary glands of patients with thyroid conditions X cells were seen; this concerned women between 43 and 68 years of age submitted to hemithyroidectomy or treated with thiouracil. In this group the intranuclear vesicles were so large as to cover almost completely the nuclei of the acidophilic cells.

In the nonendocrine group the highest number of pituitary glands containing X cells came from persons dying of neoplastic disease, especially those with a brain tumor near the sella turcica or in the middle cranial fossa which had caused compression of the pituitary body.

COMMENT

From the foregoing observations it is apparent that the occurrence of the intranuclear formations seen in the glandular epithelium of the pituitary gland is in some way related to age and gonadal function. These formations were not found in the prepubescent period and appeared most numerous, without any significant difference between male and female, in the period of sexual maturity and in a number

TABLE 3.—*Relation of Cells Containing Endonuclear Inclusions to Nonendocrine Conditions*

Subjects	Condition	Number with X Cells	Percentage with X Cells
49	Intracranial tumor	27	55
94	Neoplasms	30	32
17	Leukemia	4	24
3	Splenectomy	2	66
32	Vascular hypertension	2	6
97	Other diseases	5	5
8	Normal	0	0
Total 300		70	23

of endocrine disorders interfering with gonadal function. They were found at times in chromophilic cells, at other times in chromophobe elements with pregnancy or pregnancy-like characteristics. Gellerstedt and Lundquist [3] and Mellgren,[4] in their descriptions of hypertrophic chromophobe cells with giant nucleoli in cases of Cushing's disease (pituitary basophilism) and adrenogenital syndromes, suggested a possible relation with the "hyaline change" of the basophilic cells. That a connection existed between Crooke's hyalinosis and the X cells of my series is not likely; nevertheless, giant nucleoli often appeared in epithelial elements in the process of progressive degranulation, making identification of the hypophysial cell type extremely difficult and sometimes impossible.

In the light of the observations made by Caspersson and Santesson [5] pointing to heterochromatin (the substance entering into the constitu-

5. Caspersson, T., and Santesson, L.: Acta radiol., 1942, supp. 46.

tion of nucleoli) as the most important center of the protein syntheses and protein exchanges between nucleus and cytoplasm, the unusual richness in heterochromatin of the X cell might be considered as evidence of cellular hyperfunction.

A finding similar to that noticed in the X cells of the pituitary gland has been demonstrated in the hepatic cells of man and animals by Berg,[6] in the pineal body by Krabbe,[7] Volkmann [8] and Meyer,[9] in the cells of the pars intermedia of the pituitary gland of the mouse by Urasov [10] and Romeis,[1f] in the cells of the cortex of the adrenal gland by Schiller [11] and in the pituicytes of the human neurohypophysis by Bargmann.[12] The nucleolar patterns observed in these different structures have been generally explained on the basis of nuclear secretory activity. For the finding, closely resembling these, in the human pituitary gland, I incline toward a similar interpretation. This interpretation is further strengthened by patterns indicating a periodical emptying of the nuclear inclusion into the cytoplasmic substance. In conclusion, it is suggested that these intranuclear formations are of nucleolar origin and that they represent morphologic evidence of nuclear secretory activity.

SUMMARY

Intranuclear formations observed in the glandular cells of the human pituitary gland are described and analyzed with respect to their morphologic aspects, frequency, relation to age and disease processes, and significance.

Four hundred and four pituitary glands were examined, and evidence of such formations was found in 34 per cent. As far as sex is concerned, no differences were seen in these structures between males and females; definite relations were noticed instead with respect to age and gonadal function. The intranuclear bodies were found in both the chromophilic and the chromophobe cells but most frequently in hypertrophic chromophobe elements with pregnancy or pregnancy-like characteristics. It is suggested that these formations are of nucleolar origin and that they are the product of nuclear secretion.

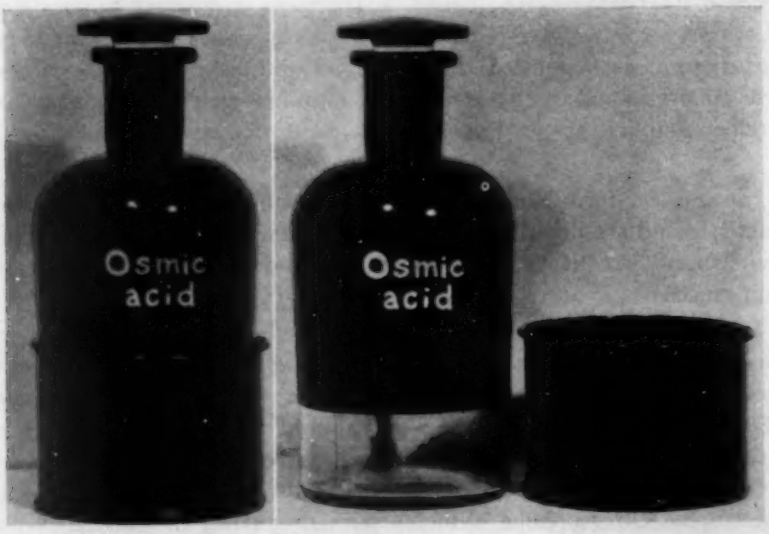
6. Berg, W.: Ztschr. f. mikr.-anat. Forsch. **35**:146, 1934.
7. Krabbe, H.: Anat. Hefte, 1916, no. 54, p. 190.
8. Volkmann, R.: Ztschr. f. Neurol. **84**:593, 1923.
9. Meyer, R.: Ztschr. f. Zellforsch. u. mikr. Anat. **25**:614, 1937.
10. Urasov, I.: Russk. Arch. Anat. **6**:149, 1927.
11. Schiller, E.: Ztschr. f. mikr.-anat. Forsch. **54**:598, 1944.
12. Bargmann, W.: Ztschr. f. Zellforsch. u. mikr. Anat. **32**:394, 1942.

Laboratory Methods and Technical Notes

IMPROVED METHOD FOR HANDLING PHOTOLABILE LIQUIDS

JOHN NICHOLS
CHAPEL HILL, N. C.

ALL USERS of photolabile liquids such as silver nitrate and osmic acid recognize the problem of preventing the deterioration due to light. Most laboratory texts say that these liquids should be kept in amber or dark-colored bottles and stored away from the light. However, if these directions are followed, there is still no certainty as to the extent of deterioration of the liquid. Important experiments may be



Painted bottle and cup for preventing deterioration of a stored photolabile liquid.

ruined as a result. The only alternative is to store the liquid in a clear glass bottle and keep it in a dark cabinet. This has many obvious disadvantages, although the condition of the liquid can be determined at a glance.

A device has been tried which seems to surmount most of the difficulties. A brief description follows.

A convenient-sized bottle with a mushroom stopper is painted jet black with a paint such as Fisher's "plicote." Care is taken not to paint any of the surface at the mouth of the bottle to avoid contaminating the liquid. A strip of adhesive tape is applied to a height of about 1 inch (2.5 cm.) from the base of the bottle

From the Department of Anatomy, University of North Carolina.

to avoid painting this area and to make a sharp border. On removal this leaves a portion of clear glass through which the liquid can be viewed. A small metal can similar to a drinking cup, which may be obtained from a 10 cent store, is also painted jet black inside and out. When the bottle is placed in the cup, after drying, there should be at least an inch of overlapping painted surfaces (figure).

The advantages offered are: (1) No light can penetrate the liquid, since the cup need not be detached from the bottle during pouring, and (2) the contents can be viewed at will by merely lifting the bottle from the cup.

General Reviews

MECHANISMS OF ABNORMAL DEVELOPMENT

III. Postnatal Developmental Abnormalities

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NEW YORK

(Concluded from Page 559)

IN the introduction to this review the fact was emphasized that no strict separation exists between developmental abnormalities of early life, on the one hand, and many of the pathologic conditions of the mature organism, on the other. One might contend that some measure of development is involved in all pathologic changes of the structure of the body, and thus bring morphologic pathology almost entirely into the scope of developmental pathology. However, the present part of this review will be confined to some of the more obvious deviations of postnatal development. It will not include those subjects which are customarily treated in a systematic manner in textbooks—for example, the structural changes resulting from inflammation or from the action of hormones. An attempt will be made to cite examples which are comparable to embryonic abnormalities mentioned in the preceding parts, in order to establish a link between the developmental pathology of the embryo and that of the adult, which is much better known. Usually they are considered from different points of view.

All those causes of structural changes which were discussed in part I with particular reference to the embryo are also effective during postnatal life. However, their relative importance is different in the two periods of life. After birth the action of intrinsic (genetic) causes, while still demonstrable in many instances, is overshadowed by that of extrinsic agents, such as mechanical forces, chemical substances, actinic rays or infectious organisms. Similarly, the mechanisms of correlation by which multiple effects are produced after one initial lesion of the

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This work was begun while the author was Fellow in Pathology at the Mount Sinai Hospital, New York.

The review of the literature was concluded in August 1946. However, many European Journals of the past few years were not available at that time, on account of the interruption of communications during the war.

embryo (see part II) are all still in existence after birth, though not with the same relative significance.

During normal development there is a gradual shift in the extent to which various correlations are active; this is reflected in a similar shift of the mechanisms of abnormal development. After birth, the basic developmental pattern of the organism is no longer subject to changes, and developmental correlations—for example, induction—are active only in the limited spheres of tissue differentiations. Genetic control of structure exists at any time, but it is not so obvious after birth. On the other hand, correlations based on organic functions, particularly those of the nervous systems and the endocrine glands, gain greatly in significance. The space allotted to the effects of various agents and correlations in the following pages will not reflect their relative significance, as those changes will be discussed in detail which are less known and which resemble the ones that are more prominent in the embryo.

HEREDITARY ABNORMALITIES

Many of the abnormal hereditary traits appear after birth, and with all methods at one's command it is impossible to detect in the newborn an abnormality which might have developed before birth. Examples to illustrate this will be cited here with the reservation that there may have been failures to discover prenatal changes. This consideration and the fact that the abnormal genotype is present in the organism at all times, no matter when it manifests itself structurally, show that there is no essential difference between hereditary changes appearing during various periods of life.

The purely descriptive literature on many of the hereditary conditions in man will not be reviewed. It may be found through references in texts of human genetics.[48]

Reference has been made previously in this review to the fact that even in the well protected embryo the manifestation of hereditary traits may be influenced by the environment. This holds, of course, to a much greater extent for postnatal life. The modifying effects of various extrinsic factors may be so great that the hereditary background is considered as merely a "disposition" toward a certain change—for example, diabetes mellitus.

Many hereditary abnormalities manifest themselves by degenerative changes which appear at a characteristic time, sometimes late in life. These have been termed heredodegenerative diseases. They are closely related to obvious malformations. It is known that such congenital defects as taillessness in mice develop in the early phase of the embryo by a very similar process of degeneration of tissues which were previously normal in appearance.

Skin.—The morphologic expression and the morphogenesis of hereditary hairlessness of man and other mammals have been examined and the literature reviewed by David.[282] The condition may appear before or after birth, depending on the genotype. Among mice with one form of dominant hairlessness, homozygous animals can be recognized at birth, whereas heterozygous animals do not appear abnormal at birth, their abnormality developing later on. According to the histologic changes, David distinguishes three forms of hypotrichosis, namely, *hypokeratotica, cystica* and *hypoplastica follicularis*. For details the original article should be consulted. Whether human baldness is comparable with these findings in animals is questionable. There seems to be a hereditary disposition, and, in addition, an influence of androgenic hormone.[498] Among the hereditary postnatal cutaneous diseases which show more or less clearly the character of developmental abnormalities are xeroderma pigmentosum, neurofibromatosis and psoriasis.

Nervous System and Sense Organs.—In the case of some abnormalities of the brain it is particularly difficult to determine the onset of abnormal changes in relation to birth, because many of the functions of that organ cannot be tested in the newborn.

Retardation or arrest of development occurs after birth in mongolism.[499] However, other manifestations of this condition date back to prenatal life.

Qualitatively abnormal development, producing a tissue structure different from any normal stage, occurs in tuberous sclerosis,[500] amaurotic familial idiocy[501] and other diseases. The exact time of onset is not known.

Numerous examples of heredodegenerative conditions of the nervous system are on record. Among these are paralysis agitans,[502] hereditary sclerosis, spinal form (hereditary ataxia, Friedreich's ataxia), hereditary chronic progressive chorea (Huntington's chorea), hepatolenticular degeneration (Wilson's disease),[45] loss of coordination in rabbits[503] and many others which may be clinically more important but which have not been thoroughly studied from the pathologic and the genetic point of view.

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498. Hamilton, J. B.: Am. J. Anat. **71**:451, 1942.

499. Benda, C. E.: Am. J. Ment. Deficiency **45**:42, 1940.

500. Globus, J. H., in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932. Yakovlev, P. I., in Blumer, G.: The Practitioners Library of Medicine and Surgery, New York, D. Appleton-Century Company, Inc., 1936, vol. 9, p. 745.

501. Globus, J. H.: J. Mt. Sinai Hosp. **9**:451, 1942.

502. Allan, W.: Arch. Int. Med. **60**:424, 1937.

503. Anders, M. V.: Am. J. Anat. **76**:183, 1945.

Several forms of hereditary metabolic deficiency affect the nervous system—for example, amaurotic familial idiocy and phenylketonuria. In the former the nerve cells accumulate the abnormal metabolic product; in the latter the mechanism by which the brain is affected is not understood.

The sense organs present excellent examples of hereditary postnatal maldevelopment. In the eyes of mice examined by Keeler [841] the differentiation of the retina was arrested shortly after birth, and this led to complete or partial absence of rod cells. A somewhat similar end result has been found in a strain of rats after degeneration of the previously well differentiated retina.[504] The latter strain, as well as a mutant of mice described by Grüneberg,[1r] shows a high incidence of cataract developing after birth.

In man, retinitis pigmentosa develops as a degeneration of the retina [505] at a varying age, depending on the mode of inheritance in the particular family.[506] Certain forms of atrophy of the optic nerves, cataract and glaucoma are also hereditary.[45]

Postnatal degenerative changes of the labyrinth account for deafness and other abnormalities of labyrinthine function in certain strains of mice. In shaker-1 mice the stria vascularis is abnormal, and as soon as the vas spirale atrophies in the usual manner, Corti's organ degenerates because it is not adequately supplied with endolymph from the stria vascularis.[507] In another mutant, the "waltzing" mouse, the inner ear is normal at birth. There is disagreement as to whether in this form there is also primary degeneration of the stria vascularis with consecutive damage of the labyrinth or primary degeneration of the ganglion spirale cochleae or of the acoustic tracts of the brain.[508]

In man a hereditary change which involves abnormal development is otosclerosis. The morphogenesis and the genetics of this condition have been reviewed in detail by Bauer and Stein.[509] Abnormal bone is formed and replaces normal bone; it forms in excessive amounts and causes fixation of the stapes. In addition there are degenerative changes in the labyrinth and its nerves. The relation of these changes to the abnormal growth of bone is controversial. Bauer and Stein conclude from investigations of the familial occurrence of diseases of the inner ear that there is a hereditary inferiority of the organ (*Organminderwer-*

504. Bourne, M. C., and Grüneberg, H.: J. Hered. **30**:130, 1939.

505. Friedenwald, J. S., and Chan, E.: Arch. Ophth. **8**:172, 1937.

506. Allan, W.: Arch. Ophth. **18**:938, 1937.

507. Grüneberg, H.; Hallpike, C. S., and Ledoux, A.: Proc. Roy. Soc., London, s.B **129**:154, 1940.

508. Grüneberg,[1r] p. 130.

509. Bauer, J., and Stein, C.: Konstitutionspathologie in den medizinischen Spezialwissenschaften, Berlin, Julius Springer, 1926, no. 2.

tigkeit), transmitted by a combination of two recessive genes. This inferiority may manifest itself either as otosclerosis or in the form of purely degenerative diseases. The histologic changes of otosclerosis have been found in cretins and deaf-mutes at a very early age.

Teeth.—The teeth present an excellent opportunity to study postnatal developmental disturbances because much of their complex development takes place after birth. Identical changes may originate before and after birth, and for this reason all malformations of teeth will be discussed jointly in various sections of this part of the review.

Weinmann [510] has classified hereditary abnormalities of enamel formation according to the developmental process involved. If the deposition of enamel matrix is inhibited, hypoplasia of enamel results; if the maturation is affected, hypocalcification is seen. Both abnormalities may also result from other than hereditary causes. Weinmann points out that the hereditary forms affect all teeth uniformly and regardless of the time of the formation of these, whereas systemic forms, caused by metabolic or endocrine disturbances, as well as those due to localized trauma, are limited to the portions of the enamel which develop at the time and the place affected.

In the mutation gray-lethal of mice, with skeletal changes which have been referred to in part II, severe abnormalities of tooth development and eruption are apparently due to a lack of bone resorption in the jaws which would normally provide the necessary space.[511] A somewhat similar hereditary malformation occurs in rats.[512] At birth the animals appear normal. Shortly after birth spicules of bone begin to encroach on the apical portions of the incisor and first molar teeth and cause severe deformity and ankylosis of the tooth germs. The incisor teeth develop into large, unerupted, tumor-like masses of dental tissues. The first molar teeth show a lesser degree of abnormality, and the second and third molar teeth, which develop later, are more nearly normal. This points to a selective action of the abnormal trait beginning shortly after birth. Only preliminary studies have been published as yet.

Skeleton, Muscles and Tendons.—Various skeletal malformations which begin to manifest themselves in the embryo (see part II) not only show after birth the effect of the prenatal abnormality but continue to follow a devious course of development. These include, among others, various forms of chondrodystrophy and the gray-lethal malformation of mice.

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510. Weinmann, J. P.: Bur **43**:20, 1943.

511. Grüneberg, H.: J. Anat. **71**:236, 1937.

512. Schour, I.; Massler, M., and Greep, R. O.: J. Dent. Research **23**:194, 1944.

A generalized malformation of cartilage which appears after birth is hereditary in the rat.[513] There is excessive growth of cartilage, and the resulting changes in the trachea and the ribs lead to emphysema and fatal pulmonary complications. Experimental transplantations have shown that the property is inherent in the tissue: grafts of cartilage from abnormal rats develop abnormally in normal hosts, and vice versa.

Postnatal hereditary abnormalities of the human skeleton have been reviewed by Aschner and Engelmann.[872a] Examples are: Legg-Calvé-Perthes disease (osteochondrosis of the head of the femur) and osteopsathyrosis (fragilitas ossium). In the muscle system there are such heredodegenerative changes as progressive muscular atrophy and peroneal atrophy (progressive neuropathic muscular atrophy). Concerning tendons, Dupuytren's contracture of the palmar fascia might be mentioned.

Other Organs.—Imperforate vagina occurs as a hereditary trait in mice.[1r] The abnormality manifests itself at puberty, when the normal vagina acquires a lumen. This process fails in the abnormal mice, and it cannot be initiated by estrogenic treatment. If the vagina is opened surgically, the female is fertile. However, the vagina retains a tendency to close, in contrast to the normal vagina, which tends to open again if it is closed surgically.

Another strain of mice shows, beginning at the age of 3 to 4 months, severe adenomatous thickening of the mucosa of the pyloric portion of the stomach.[514] This observation leads into a field which should be of considerable medical importance, namely, hereditary differences in the old age changes of various organs. The following examples are discussed more in detail in Grüneberg's book on genetics of the mouse.[1r] Gorer [515] found in each of three strains of mice which he examined a specific change in the kidneys in advanced age. One strain showed metaplasia of the parietal layer of Bowman's capsule more frequently than did other mice. In another strain necrotic lesions of the papillae appeared, with consecutive dilatation of tubules. The third strain showed hyaline degeneration of the connective tissue framework. It is highly significant that these changes were not the result of diseases caused by extrinsic agents but were rather the expression of the genetic constitution, either alone or, as a predisposing factor, in combination with extrinsic influences which would otherwise not have this effect. The changes just mentioned are obviously related to the so-called heredodegenerative diseases which have just been referred to and to prenatal malformations which develop by degeneration of previously normal-appearing parts.

513. Grüneberg, H.: Proc. Roy. Soc., London, s.B **125**:123, 1938. Engel, S., and Grüneberg, H.: J. Genet. **39**:343, 1940.

514. Andervont, H. B., and Stewart, H. L.: Science **86**:566, 1937. Stewart, H. L.: J. Nat. Cancer Inst. **1**:489, 1941.

515. Gorer, P. A.: J. Path. & Bact. **50**:25, 1940.

There are interstrain differences in the occurrence of the so-called brown degeneration of the adrenal glands and in the structure of the follicles of the thyroid gland and the incidence of goiter. Also in mice there are genetically determined differences in the time of appearance of old age changes of bones and joints.[516]

Progressive facial hemiatrophy of man has been considered by Wartenberg [65d] as a heredodegeneration, possibly mediated by unilateral changes in the nervous system.

MECHANICAL AGENTS

Mechanical injury in the widest sense accounts for many structural changes. In most cases the abnormality is just the result of an elimination or a disfiguration of parts plus the usual processes of wound healing; these cases offer nothing to be discussed here. There are, however, instances in which more complex developmental processes are modified by mechanical injury. Among these is the growth of tissue displaced by trauma.

Few noncancerous tissues of the mature organism grow for a long period when transplanted to abnormal locations, unless special precautions are taken as in surgical grafting. Several cases are on record in which multiple nodules of splenic tissue grew on the peritoneum after the spleen had suffered traumatic rupture.[517] Another example, namely, endometriosis, is controversial. Some authors assume that viable endometrial tissue is displaced either by being regurgitated through the tubal ostiums or in the course of surgical procedures [518] as suggested by the appearance of endometriosis in surgical scars. On the other hand, there are sites of endometriosis which are not explained by either of these theories. It has been demonstrated that all known locations of endometriosis can be accounted for embryologically without recourse to mechanical displacement (see part II). This, of course, does not disprove the claim that mechanical transportation of tissue has occurred in some cases.

It may appear strange at the first glance that bone, one of the tissues of the body which mechanically are most rigid, should react most actively to mechanical influences by developmental changes. Yet it is this very rigidity which makes developmental changes of the structure necessary in cases in which other tissues, such as muscles, tendons or ligaments, could adapt themselves by means of their flexibility. The arrangement of the trabeculae and the haversian systems of bone is adapted to mechanical

516. Silberberg, M., and Silberberg, R.: Am. J. Anat. **68**:69, 1941.

517. Buchbinder, J. H., and Lipkoff, C. J.: Surgery **6**:927, 1939. Jarcho, S., and Anderson, D. H.: Am. J. Path. **15**:527, 1939.

518. Sampson, J. A.: Arch. Surg. **3**:245, 1921. Wespi, H. H., and Kletzhändler, M.: Monatschr. f. Geburtsh. u. Gynäk. **111**:169, 1940.

strain, and any change occurring in the mechanical conditions of the environment is immediately followed by resorption and rebuilding of parts of the bone. This is well demonstrated by the minute structure of bones with abnormal curvatures.[519] Pressure causes resorption of bone not by crushing or destroying the tissue but by the action of osteoclasts. This explains, for example, why the bodies of vertebrae are less resistant to the pressure exerted by an aortic aneurysm than are the intervertebral disks. Mechanical pull, on the other hand, causes overgrowth of bone, as seen at the points of insertion of tendons.

Müller [520] has presented in book form the developmental physiology and pathology of bone, with particular reference to the differentiation of inherent and extrinsic factors of development. Iselin [521] has pointed out how the principles of developmental mechanics should be used as a basis for rational therapy in orthopedics.

Displaced tissues of the mature organism may in a few instances act on their new surroundings as inductors. The best studied example is that of bone formation inducted by transplants of mucosa of urinary passages.[454] It has been found that the connective tissue reacts by formation of bone only in certain locations (e. g., the abdominal wall). In other locations (e.g., the liver, the spleen, or the wall of the stomach) a connective tissue capsule develops around transplants of bladder mucosa, but no bone. This has been compared with similar observations in embryologic experiments demonstrating that only part of a morphologically uniform tissue is able to react to certain inductions.[522] The fact that alkaline phosphatase is present in the transplanted epithelium has led to the suggestion that phosphatase diffusing into the connective tissue might be the cause of ossification. This has been ruled out not only by the negative findings in certain areas, even though connective tissue was present and the epithelium contained phosphatase, but also by the negative results observed in all locations of other transplanted phosphatase-containing epitheliums. The phosphatase which is present in areas of ossification induced by bladder mucosa is produced by fibroblasts as the first known response to the induction, before routine staining methods show any change in these cells.[522]

THE INFLUENCE OF CHEMICAL AGENTS

Certain substances which may or may not be essential for normal development and function in certain amounts are poisonous when present

519. Landauer, W.: Arch. f. Entwcklngsmechn. d. Organ. **115**:911, 1929. Sternberg, H.: Ztschr. f. orthop. Chir. **63**:387, 1935. Murray, P. D. F.: Bones, London, Cambridge University Press, 1936.

520. Müller, W.: Die normale und pathologische Physiologie des Knochens, Leipzig, Johann Ambrosius Barth, 1924.

521. Iselin, H.: Schweiz. med. Wchnschr. **14**:465, 497 and 536, 1933.

522. Gomori, G.: Am. J. Path. **19**:197, 1943.

in excessive amounts. In many instances their action, though morphologically specific to some degree, is of little interest from the standpoint of this review. Among those substances which have a definite influence on development are carcinogens, which will be mentioned in a later section dealing with cancerous growth, as well as others which affect normal developmental processes. In addition, deficiencies of some inorganic compounds may disturb development.

Other groups of substances are specifically related to developmental processes. Vitamins are essential for normal development; their lack or, in rare cases, their excess causes disturbances. Hormones are elaborated in the body, and one of their principal functions is the control of certain phases of morphogenesis (see also parts I and II). It is obvious that excess or lack of these "messengers" will profoundly influence development and maintenance of structure. Most of the well studied morphologic sequelae of abnormal levels of hormones and vitamins have been treated systematically in many monographs and textbooks and will therefore not be dealt with here. Only a few examples will be given.

The continuously growing teeth of rodents are a good object for the study of the effect of all of the aforementioned types of chemically caused disturbances. The formation or the maturation of the enamel, the formation of dentin, the eruption of the tooth and the structure of the socket may be affected singly or in various combinations.

Deficiency of magnesium in the diet of rats reduces the rate of eruption. Dentin formation is also reduced, and ceases completely in focal areas.[523] An excess of fluorine compounds reduces the rate of eruption and causes defective calcification of dentin and enamel. If large doses are given, enamel formation suffers by a shortening of the appositional life span of the ameloblasts. Changes observed in human fluorosis are in some respects similar to these experimental results.[524] Strontium and manganese compounds cause hypoplasia of enamel but in somewhat different manners, as has been shown by Wessinger and Weinmann [525] in histologic studies and illustrative diagrams.

The effects of vitamin deficiencies on postnatal developmental processes in man and in experimental animals has been summarized by Wolbach and Bessey [526] in a review in which much information and a comprehensive list of references may be found. A smaller volume of data on hypervitaminoses is also contained in that review. At an earlier date, Wolbach [527] pointed out that studies of vitamin deficiencies "may be

523. Gagnon, J.; Schour, I., and Patras, M. C.: Proc. Soc. Exper. Biol. & Med. **49**:662, 1942.

524. Schour, I., and Smith, M. C.: Publication 19, American Association for the Advancement of Science, 1942, p. 32.

525. Wessinger, G. D., and Weinmann, J. P.: Am. J. Physiol. **139**:233, 1943.

526. Wolbach, S. B., and Bessey, O. A.: Physiol. Rev. **22**:233, 1942.

527. Wolbach, S. B.: Science **86**:569, 1937.

of value as premises in problems, hitherto approached only by the methods of experimental embryology . . . ". Wolbach and Bessey classify the morphologic manifestations of vitamin deficiencies as follows: (1) diffuse consequences expressive of inanition; (2) effects common to several deficiencies, especially degenerations of the nervous system and, with qualifications regarding fine details, lesions of the skin; (3) degenerative changes characteristic in kind and distribution, best illustrated by the cerebral lesions of thiamine deficiency and the degeneration of skeletal muscles and embryonal tissues observed in vitamin E deficiencies; (4) initial specific effects exhibited by striking changes of structural patterns, outstanding in relation to vitamins A, C (ascorbic acid) and D. As was found earlier in the present review, postnatal maldevelopment is particularly obvious in the skeleton and the teeth, and much of present information on vitamin deficiencies concerns these structures.

One of the most important effects of vitamin A deficiency is seen in the keratinizing stratified epithelium formed in many mucous membranes which normally have a different lining. In the continuously growing teeth of rodents there are atrophy and keratinizing metaplasia of the enamel epithelium with consecutive irregularity, and finally cessation of dentin formation. If the vitamin deficiency is not complete, irregular remnants form tumor-like aggregates of dental tissues.[528]

According to Johnson,[529] vitamin A deficiency produces in the eyes of rats degeneration of the retina and rosette formation. This is of particular interest because, as has been reviewed in part II, rosettes develop in the embryo as abnormalities during the differentiation of the retina. They can apparently also arise secondarily after normal differentiation has been completed. The mechanism is probably one of rearrangement of the remaining cells after extensive degeneration.

Skeletal growth and development are inhibited in avitaminotic young animals, and the ensuing limitation of space in the cranial cavity and the spinal canal causes herniations and other disturbances of the nervous system. An excess of vitamin A produces osteoporosis and decalcification of bone, leading to multiple fractures. Osteoporosis is most marked in areas in which bone is normally being remodeled at the time of the disease.[526]

Deficiencies of vitamins of the B complex are not followed by such characteristic and generalized changes as is exemplified by the epithelial metaplasia of vitamin A deficiency. Most of the pathologic aspects are those of degeneration, with which one is not concerned here. Examples of abnormal development that probably is a consequence of such degen-

528. Burn, C. G.; Orton, A. U., and Smith, A. H.: Yale J. Biol. & Med. **13**:817, 1941.

529. Johnson, M. L.: J. Exper. Zool. **81**:67, 1939.

erative changes are imperfect growth of hair, the hyalinization and vascularization of the tunica propria of the cornea seen in riboflavin deficiency and the cirrhosis following fatty changes of the liver in choline deficiency.[526]

Vitamin C produces a characteristic generalized change in the supporting tissues by a "failure of formation and maintenance of intercellular materials".[526] All the changes found in human scurvy and in deficiency experimentally produced in the guinea pig are explained on this basis. The viability of the cells of the affected tissues is not diminished. The formation of bone and dentin is abnormal and finally ceases. Osteoblasts and odontoblasts become indistinguishable from fibroblasts. Wound healing is poor. Wassermann [530] shows that in the teeth of guinea pigs enamel formation ceases in areas exactly corresponding to those of absence of dentin, and he explains this by stating that the ameloblasts depend on the presence of dentin for their normal function.

According to Wolbach and Bessey, vitamin D deficiency acts not directly on the bony structures which show the striking effects but on the absorption of calcium and phosphate, which becomes inadequate for the proper calcification of tissues. This affects the cartilage of the epiphyses of growing bones and thus prevents the normal changes preceding the destruction and bony replacement of that tissue. Consequently the cartilage accumulates in the epiphyses. Another feature of rickets is the deposition of much uncalcified osteoid tissue instead of bone and the resorption of some of the preexisting bone. Osteoid tissue is highly resistant to osteoclastic resorption. In older individuals, in whom epiphysial growth no longer occurs, bone tissue is gradually replaced by solid masses of soft osteoid tissue (osteomalacia). The changes in teeth have repeatedly been examined. Weinmann and Schour [531] report on rachitic changes of the continuously growing teeth and the alveolar bone of rats and on their modification by various agents. They found, in contrast to some earlier investigators, that the formation and the maturation of enamel were unaffected. The formation and the calcification of dentin were retarded. In addition there were qualitative abnormalities in the dentin. The alveolar bone showed the well known lack of calcification of newly formed bone tissue and failure of this osteoid tissue to be resorbed. This produced a distorted pattern of growth. The increased resistance of osteoid tissue to resorption remained in evidence after treatment with parathyroid extract. As in other instances, the rachitic changes were reversed by starvation or by the administration of sodium phosphate solution or viosterol.

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530. Wassermann, F.: J. Dent. Research **23**:463, 1944.

531. Weinmann, J. P., and Schour, I.: Am. J. Path. **21**:821, 833, 857, 1047 and 1057, 1945.

One of the principal fields of hormone action is the regulation of morphogenesis and structure. The amount of work done in this field is so large, and it has so often been reviewed, that it will not be treated here. Texts of physiology or pathology, as well as specialized accounts of endocrinology, are available for reference. Endocrine influences on tooth development which are not described in detail in many of these works have been reviewed by Schour and Massler [532]; references to original reports may be found there.

A field in which development under hormonal control is particularly active after birth is that of the sex organs and sexually differentiated traits. This is also of great interest, because it demonstrates the interaction of genetic and hormonal controls of development. Certain abnormalities, such as those induced by hormones released by adrenal cortex tumors, are essentially similar before and after birth. Some fundamental aspects of these problems have been discussed in parts I and II.

Inductors are substances of eminent importance in structural development. They differ from hormones in the manner in which they reach their destination in the body: They are directly transmitted from one tissue to an adjacent one and are not distributed to the entire body by way of the blood stream. Abnormal induction will therefore occur when tissues are in contact with one another in a manner in which they would not be in the normal body. This may be brought about by mechanical dislocation of the inductor or of its substrate, as has been illustrated in a previous section with reference to bone induced by transplants of bladder mucosa.

THE EFFECTS OF INFECTION

I shall not discuss here the effects of protracted infectious diseases on general growth and development. The impairment of these processes is not so much a specific effect of the infectious agent as the result of nonspecific unfavorable circumstances, such as malnutrition or fever.

If infection provokes the growth of a tissue which would not usually be present, this is usually in the form of a granulation tissue. A discussion of inflammation and the formation of granulation tissue is not within the scope of this review, even though certain developmental mechanisms are involved in the differentiation of cells in this process and in the determination of the pattern of the lesion. However, brief mention should be made of what has sometimes been called specific granulation tissue. Granulomas of a definite and complex structure are in many cases so characteristic of a causative agent that their presence is considered sufficient evidence for diagnosis in routine practice

532. Schour, I., and Massler, M.: J. Am. Dent. A. **30**:595, 763 and 943, 1943.

of pathology, without the need for demonstrating the causative agent itself. The question thus arises whether these granulomas should be considered as specific developmental responses to the presence of certain micro-organisms. If one analyzes these granulomas, one finds that they are composed of a few elements of nonspecific reaction—for example, to foreign bodies. Various combinations of these elements and the peculiar distributions and biologic properties of the micro-organisms involved account for the characteristic appearances which granulomas may have. This has been analyzed in great detail in the case of tuberculosis, where various fractions of the substance of the bacilli have been isolated and the reactions of mammalian tissues to their presence observed. This work, as well as a large volume of information concerning tissue reactions to various injurious agents, has been reviewed by Forbus.[533]

In a few instances infection provokes structural alterations which do not fall into the group of inflammatory reactions. One example is the contagious pulmonary adenomatosis of sheep (jagziekte); the exact nature of the infectious agent has not been determined.[534] The lungs show an adenomatous nodular growth of columnar epithelium lining the spaces.

In some persons the teeth show evidence of aplasia of the enamel during a limited period of development, and it has been suggested many times that various diseases, among them infections, may be the cause. Sarnat and Schour [535] found no definite evidence of infectious diseases as the cause of chronologic aplasia of enamel in a series of 60 cases, but they admit that this relationship may occasionally exist. The effect on the teeth is a nonspecific one and does not indicate that the dental tissues were actually infected. The same probably holds for the observations of Kreshover,[536] who reported that in experimental tuberculosis of laboratory animals changes occur similar to the aforementioned chronologic aplasia in man.

DEVELOPMENTAL ASPECTS OF CANCER

The most striking peculiarity of cancer is a developmental abnormality concerning its structural differentiation as well as its rate of growth and its relation to adjacent structures. Many data relating to these and other aspects of the problem of cancer have recently been reviewed by Furth,[68b] and many original reports, as well as previous and more specialized reviews, are listed there.

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533. Forbus, W. D.: Reaction to Injury: Pathology for Students of Disease Based on the Functional and Morphological Responses of Tissues to Injurious Agents. Baltimore, Williams & Wilkins Company, 1943.

534. Dungal, N.: Am. J. Path. **22**:737, 1946.

535. Sarnat, B. G., and Schour, I.: J. Am. Dent. A. **28**:1989, 1941; **29**:67, 1942.

536. Kreshover, S. H.: J. Dent. Research **21**:27, 1942; **23**:231, 1944.

Investigators know that all types of causes of abnormal development which have been discussed with regard to embryonic malformations in part I of this review may also produce cancer under proper conditions. They include hereditary factors and influences of the environment of a mechanical, chemical, radiant, thermic or infectious nature. As is often the case in embryonic maldevelopment, several of these factors (e.g., hereditary and environmental ones) may combine their effects also in carcinogenesis.

Hereditary factors may appear in two ways. In one of these, cancer develops in individuals carrying a certain gene or combination of genes either by virtue of these genes alone or when an environmental agent is added. In the latter event the genetic constitution determines what is often called the susceptibility to the environmental agent. The second way in which genes are concerned with cancer relates more directly to the cancerous tissue itself; this is somatic mutation. The fact that the essential abnormality is apparently passed on from one cancer cell to its descendants without the necessity of the continued presence of a carcinogenic agent has led many workers [537] to the conclusion that cancer cells are genetically different from the cells of their host, which constitutes a somatic mutation. In support of this hypothesis it has been pointed out that agents which are known to increase the mutation rate (e.g., roentgen rays) are also carcinogenic. Strong [68c] reports that one of the most commonly used chemical carcinogens, 20-methylcholanthrene, also increases the rate of germinal mutations.

Among environmental agents, virus infection has been held by Oberling [538] to be responsible for all cancers. Actually, it has been found in regard to certain cancers of the mammary glands of mice, which were previously believed to be purely hereditary, that one of the causative agents is a substance transmitted to the young by the mother's milk.[539] The properties of this substance resemble those of a virus as far as they are known. However, Oberling's theory has not been accepted to its full extent.

The nature of the basic abnormality of cancerous growth has been defined in various manners, each depending largely on the line of approach of the investigator. Pathologists, biochemists, immunologists, geneticists and others have found differences between cancerous and noncancerous tissue. Within the scope of the present review are only

537. (*a*) Bayne-Jones, S., and others: Pub. Health Rep. **53**:2121, 1937. (*b*) Berrill, N. J.: Physiol. Rev. **23**:101, 1943. Furth.[68b]

538. Oberling, C.: The Riddle of Cancer, New Haven, Yale University Press, 1944.

539. Bittner, J. J., in Research Conference on Cancer, American Association for the Advancement of Science, 1945, p. 63. Shimkin, M. B., and Andervont, H. B.: ibid., p. 97.

those facts and theories which relate to cancer as a phenomenon of abnormal development. All those[540] who have discussed in recent years the problem of cancer from the point of view of developmental mechanics have justly emphasized the obvious fact that cancer cells are not subject to those regulatory mechanisms which normally control growth and differentiation and assure that each part keeps within the limits of the structural pattern of the entire organism. There are indications that in some cases cancerous growth is initiated when a tissue is removed from the regulatory influences of its surroundings. This interpretation might be used in the cases in which carcinoma originates in repeatedly transplanted mammary glands[541] or in those in which explanted and reimplanted cells show malignant growth.[542] Lack of proper regulation may also be claimed for cancer developing in regenerating tissue—for example, in cirrhotic livers, or after subtotal orchiectomy in birds.[543]

In the great majority of instances, however, there is no evidence that a lack of organic control is the cause of cancer. It is quite possible, if not probable, that the lack of response to existing control mechanisms is a fundamental property of cancer cells rather than the cause of their cancerous behavior. The possibility that this change within the cells may be determined by genes, has already been mentioned.

Not in all cases of cancer is there a complete lack of response to normal controls. A well studied example of the effectiveness of such control and its therapeutic application is the carcinoma of the prostate. In many cases the tumor shares with its parent tissue the susceptibility to hormonal influences and regresses when the level of testicular hormone is lowered—for example, by orchiectomy.[544]

The function of cancer cells is well preserved in many cases. These cells may elaborate secretions (e.g., mucus, bile) or hormones, or undergo cornification, or produce a characteristic ground substance (e.g., bone). The function may be much better developed than the structural differentiation would make one believe; this has been observed in hormone-producing cancers.

The resemblance of cancerous and embryonic cells is superficial. It consists in rapid multiplication and a low degree of structural differentiation. On the other hand, one of the fundamental properties, namely, the lack of organization, distinguishes cancer very definitely

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540. Waddington, C. H.: Nature, London **135**:606, 1935. Needham.[4] Berrill.[537b]

541. Fischer, A.: Am. J. Cancer **31**:1, 1937.

542. Earle, W. R., in Research Conference on Cancer, American Association for the Advancement of Science, 1945, p. 139.

543. Champy, C., and Lavedon, J. P.: Compt. rend. Acad. d. sc. **207**:99, 1936.

544. Huggins, C.; Stevens, R. E., and Hodges, C. V.: Arch. Surg. **43**:209, 1941.

from embryonic tissue, which shows the effect of organizing influences to a high degree. In cancer, both the rapid growth and the poor differentiation are related to the lack of control. The poorly differentiated cancer cell is too abnormal to differentiate properly; the embryonic cell, on the other hand, is normal and may have several possible ways of differentiation depending on its environment. The cancer cell therefore differs more from the embryonic cell with its multiple potencies for differentiation than from the adult cell which may have undergone a reduction of these potencies and is less accessible to organizing influences. Therefore, a poorly differentiated cancer cell should be called anaplastic and not embryonic.[545]

The just mentioned resemblance of embryonic and cancerous cells accounts for the great interest which many workers have taken in the rare cases of cancer of the fetus. The rarity alone is significant, and it illustrates the statement that actually embryonic and cancerous cells are not closely related. After careful scrutiny, Wells[546] reviews and discusses a limited number of unquestionable cases of prenatal cancer. Among these are no typical cases of carcinoma. A more recent report of bilateral ovarian carcinoma occurring in a premature infant[547] must be discarded; it deals with normal ovaries in which some of the sex cords are not yet divided into follicles. These cords and their transitions into follicles were described as carcinoma cords arising from follicles. I have seen many similar ovaries in newborn infants.

In conclusion, the meager information concerning the fundamental developmental aspects of cancerous growth can be summarized as follows: Developmental abnormalities, particularly the lack of integration into the pattern of the organism and the poor structural differentiation, are properties rather than causes of cancer. As Berrill[887b] points out, the incompleteness of present knowledge of the fundamental mechanisms "is due to the standards of reference also being problems as challenging as malignancy itself." Among the possible causes of cancer which include all types of agents that may produce malformations, two are now being investigated as more universally active than others: somatic mutation and virus infection.

There is one fundamental difference between cancer and most of the other developmental abnormalities which have been discussed in this review. In the latter, the teratogenic factors act during a short period of development and produce certain changes. Subsequently, development proceeds by normal mechanisms, and the result is abnormal only because the substrate of these mechanisms is abnormal, owing to

545. Ewing, J.: Neoplastic Diseases: A Treatise on Tumors, Philadelphia, W. B. Saunders Company, 1941. Gruenwald.[482]

546. Wells, H. G.: Arch. Path. **30**:535, 1940.

547. Ziegler, E. E.: Arch. Path. **40**:279, 1945.

earlier changes. In cancer, on the other hand, the abnormal mechanism is perpetually active; it has been mentioned that this is one of the facts suggesting a genetic change in the cancer cells by somatic mutation.

CLOSING COMMENT

The present review is based on excerpts from a huge volume of pertinent information scattered throughout the literature of all divisions of medicine, as well as zoology, genetics, veterinary medicine, agricultural research and other fields. In the past, research in developmental pathology has been conducted from many different points of view, and few workers in the field have had a knowledge of previous work in other parts of this discipline, and of some of the basic problems involved. Developmental pathology deserves to be recognized as a portion of biology and pathology in which much has already been accomplished. If further work is to be successful, and if it is to be done in a rational manner, it is necessary to take stock now and endeavor to correlate whatever information is at hand. The present review is an attempt in this direction.

It is obvious that all those disciplines which have contributed to developmental pathology will in turn benefit from the progress made in this field. This holds particularly for practical medicine. Only a few years ago the knowledge of abnormal development had little of practical value to offer to the physician. This changed when within a few years great advances were made in such subjects as erythroblastosis fetalis, cystic fibrosis of the pancreas, malformations caused by rubella during pregnancy and the surgical treatment of several previously hopeless malformations. A stage has been reached in which developmental pathology is bound to make valuable contributions to practical medicine.

Obituaries

GEORGE HOWITT WEAVER, M.D.

1866-1947

George Howitt Weaver was born Oct. 22, 1866, in Waukesha County, Wisconsin. He died at Wilmette, Ill., on April 19, 1947. Dr. Weaver was a graduate of Carroll College, Waukesha; he was of the class of 1889 at Rush Medical College. On completing an eighteen months' internship in Cook County Hospital he engaged in general practice with Dr. Charles Warrington Earle. After Dr. Earle's early death he continued practice for a few years. In the early 90's he spent several months in the study of pathology and bacteriology at the Johns Hopkins Hospital, later carrying on work of the same character with Dr. Hektoen in the John McCormick Institute for Infectious Diseases. From 1905 to 1914 he was attending physician at Cook County Hospital, where he had charge of wards for infectious diseases. In Rush Medical College he was a member of the department of pathology from the time it was organized in 1894, and conducted the early laboratory courses in bacteriology. In 1918 he was appointed professor. In 1902 he joined the staff of the John McCormick Institute for Infectious Diseases and from 1913 to 1933 he served as physician in charge of the Durand Hospital of this institute.

Dr. Weaver was a member of many medical societies: American Medical Association, Illinois State Medical Society, Chicago Medical Society, Chicago Pathological Society, American Association of Pathologists and Bacteriologists, Association of American Physicians and the Institute of Medicine of Chicago. He was the efficient secretary of the Chicago Pathological Society for twenty-five years and also the first secretary of the Society of Medical History of Chicago.

From 1890 to 1933 he made frequent contributions to medical journals, some 65 articles being listed in his bibliography. The earlier articles were reports of cases, their clinical course, diagnosis and pathology. Later articles were especially concerned with the bacteriologic and immunologic aspects of disease and with experimental investigations. He made important pioneer clinical studies on serum disease. Especially valuable were his "Reports of the Durand Hospital of the John McCormick Institute for Infectious Diseases." In these reports, which were made at five year intervals, he embodied not alone much worth while statistical information and results of a scientific study of cases, but dwelt on practical details: the need of a period of observation

before admission of the child to the ward and meticulous efforts to avoid mixed infections; the advantage of the face mask; the extreme importance of careful nursing.

Dr. Weaver was a bibliophile in the true sense, a keen judge of the historic value of a book, a pamphlet or an old college catalogue. His papers on biographic and historical topics are of great interest. He

GEORGE HOWITT WEAVER, M.D.
1866-1947

possessed many items concerned with medical biography and the early history of medical education in Illinois and the West. Many of these items were purchased by Vanderbilt University. A large collection made on behalf of the Society of Medical History of Chicago has gone to the John Crerar Library.

Dr. Weaver was quiet and unassuming. He was of a scholarly bent of mind, activated by a spirit of investigation. In judgment he was well poised; in expression of opinion, clear and accurate. He possessed intellectual as well as ethical honesty. He was fond of flowers and travel; he enjoyed intercourse with congenial friends, to whom he revealed a hitherto unsuspected delicious sense of humor. For several years a distressing physical ailment which he bore uncomplainingly kept him from active participation in medical affairs.

In June 1901 he married Carrie Earle, who survives him.

George H. Weaver will be enrolled as a benefactor of the Institute of Medicine of Chicago, for he willed to it a fund for the endowment of a Charles Warrington Earle Lectureship.

JAMES B. HERRICK.

Books Received

COLLOID SCIENCE: A SYMPOSIUM. Contributors: E. K. Rideal, A. E. Alexander, D. D. Eley, P. Johnson, F. Eirich, R. F. Tucket, J. H. Schulman, M. P. Perutz, G. S. Adair, G. B. B. M. Sutherland and R. R. Smith. Pp. 208, illustrated. Price $6. Brooklyn: Chemical Publishing Company, 1947.

This small book is a collection of condensed elementary lectures, each terminated by a few well selected authoritative references. The lectures deal principally with special aspects of thermodynamics, physical chemistry and organic chemistry as these are used in the development of theory and experiment in colloid science. Emphasis is placed especially on disperse systems, macromolecules and interfacial films composed of one or more layers of molecules. Several important and interesting phases of colloid science are mentioned only briefly or not at all. In general, the book may be regarded as worth while only for those who are wholly unacquainted with the theory, the scope and the methods of colloid science or, more particularly, of chemistry and physics as these are applied to the development of knowledge of the structure and the behavior of disperse systems. Those who are interested in colloid science as applied to biologic systems might find great satisfaction in reading the chapter concerned with the manner in which fat is absorbed from the intestinal lumen.

L'HYPERINSULINIE: LES ÉTATS DE SURACTIVITÉ FONCTIONNELLE DU PANCRÉAS ENDOCRINE EN MÉDECINE EXPÉRIMENTALE ET EN CLINIQUE. By Marcel Sendrail, professeur de pathologie générale à l'Université de Toulouse. Pp. 256, illustrated. Price 500 francs. Paris: Masson et Cie, 1947.

This monograph presents a well documented review of the various states in which hyperactivity of the endocrine portion of the pancreas is involved. The subject is divided into seven sections under the headings "Biological Concept of Hyperinsulinism," "Experimental Hyperinsulinism," "Clinical Aspects," "Diagnostic Methods," "Causes of Spontaneous Hyperinsulinism," "Extrainsular Hyperinsulinism" and "Therapeutic Problems."

Because of its abundant reference material, especially on the clinical aspects, the book is a valuable one for those clinicians and physiologists who are particularly interested in metabolic and endocrine problems. It contains, however, points of view and deductions from data which are open to much criticism. Often one case report serves the author for a generalization. The discussion of the nervous control of the secretion of insulin is given more prominence than the available knowledge justifies. Hypoglycemia is frequently equated with hyperinsulinism without evidence that the islets are hyperactive.

Despite these critical strictures, the monograph remains a valuable source book.

CALCIFIC DISEASE OF THE AORTIC VALVE. By Howard T. Karsner, M.D., and Simon Koletsky, M.D., Institute of Pathology, Western Reserve University, and the University Hospitals of Cleveland. Pp. 107, with 24 illustrations. Price $5. Philadelphia: J. B. Lippincott Company, 1947.

Based on a thorough analysis of previous studies of calcific disease of the aortic valves and on a careful study of 200 cases observed by the authors, much valid and instructive information is presented in this monograph. There are many excellent photographs, as well as informative tables and charts. Terse and lucid, it is a classic addition to the literature of calcific disease.

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Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.